967.6

New Zealand Journal of Agricultural Research

VOLUME 2 No. 2

APRIL 1959

COMMONWEALTH MYCOLOGICAL INSTITUTE
24 JUN 1959

NOTICE TO CONTRIBUTORS

General

Papers published, or offered for publication, elsewhere are not acceptable. Nevertheless, publication elsewhere of an abstract or extended summary does not preclude publication in full in this journal.

Typescript

The original and one carbon copy are required, on one side only of foolscap paper, double spaced, with a left-hand margin of at least one inch and a quarter. Reasonably heavy good-quality paper should be used; flimsy paper delays the machine operator and consequently increases the cost of printing.

A brief summary is required, at the beginning of the paper. It should indicate the scope of the paper and give the principal results, and should be suitable for reproduction by abstracting journals as it stands.

All matter to be printed in italic type (e.g. generic and specific names) must be underlined.

Tables

Tabular matter costs more to print than letterpress and should therefore be kept to a minimum, bearing in mind, however, that a well planned table can take the place of a much greater area of letterpress.

Wherever possible, tables should be arranged to fit upright on the page, not sideways. They will be printed in 8-point type, and must be limited in size to fit the printed page, i.e. $4\frac{1}{4} \times 7$ in. maximum.

All tables, however brief, should be numbered, in arabic numerals. Numerous very small tables should be avoided.

Titles should be as brief as possible.

Units of measurement should be placed in parenthesis at the head of the column, not in the body of the table. Descriptive notes should be kept to the minimum, and abbreviations used wherever possible. For abbreviations, etc., the usage followed is that of British Standard 1991, Letter Symbols, Signs, and Abbreviations. Part 1. General.

Figures

These are to be numbered consecutively in arabic numerals, regardless of whether they are half-tones (photographs) or line blocks (graphs, etc.). Each must be referred to in the text, and only such figures as are essential to elucidate the text can be published. Graphs showing the same data as tables are not accepted.

The author's name, the title of the paper (abbreviated), and the figure number, should be written lightly in soft pencil on the back of each figure.

The maximum size for any figure is $4\frac{1}{4} \times 7$ in., less the space required for the caption.

Photographic prints should be on glossy paper, and may be "same size" or larger than the printed picture. Components of a composite figure should be firmly mounted on white card, and lettered as required A, B, C, etc.

Graphs should preferably be drawn about twice the final size, with the curves evenly inked throughout. Great care should be taken to ensure that all detail, especially lettering, is of such size as to be clearly legible when reduced to the final size. If he wishes, the author may insert lettering in pencil for re-draughting in this office.

If the scale is important, it should be shown on the figure, or, alternatively, the magnification or reduction may be stated in the caption, taking into account the printing reduction.

The figure number should not appear on the figure itself, only in the caption. Captions for all figures should be typed together at the end of the paper.

References

In the text, citations are to be by author's name and year, e.g. "(Brown 1957" or ". . . . as stated by Brown (1957)". The list of references at the end of the paper is to be arranged in alphabetical order of authors' surnames. Titles of papers cited may be included or omitted; if included, all words other than conjunctions and pronouns are to have capital initial letters. Titles of periodicals are to be abbreviated in accordance with the "World List of Scientific Periodicals" 3rd ed. 1952. The title of the periodical and the volume (and part) number are to be underlined for italics, but not the page number(s). Titles of books are to be in inverted commas and not underlined; the name and location of the publisher are to be given. For arrangement, punctuation. etc., of references, see examples in this issue.

NEW ZEALAND JOURNAL
of
AGRICULTURAL RESEARCH

Department of Scientific and Industrial Research, Wellington
Editor: M. A. Black.

VOL. 2. APRIL 1959 Number 2

BARLEY QUALITY TESTS

I. VARIETY TRIALS

By ROSA STERN, H. J. Kearns Ltd., Christchurch, and G. M. WRIGHT, Crop Research Division, Department of Scientific and Industrial Research, Christchurch.

(*Received for publication, 3 December 1958*)

Summary

Quality determinations were made on samples of barley from drilled variety trials sown at the Crop Research Division, Lincoln, in 1955 and 1956. There were significant seasonal differences in husk percentage and in total β-amylase. Varietal differences in percentage extract (Pawlowski's method) were established; the highest extract was given by Carlsberg, followed by Piroline, Kenia, Research, Maythorpe and Proctor. These results are not in agreement with those obtained elsewhere by the standard method of the European Brewery Convention. To provide the different types of malt required in New Zealand, several varieties should be grown: Research and Kenia have an established place in New Zealand farming, and the further investigation of the performance of Carlsberg and Maythorpe is justified.

INTRODUCTION

The only information published, on the quality of locally grown samples of the varieties of malting barley under test in New Zealand, refers to the two standard commercial varieties, Research and Kenia (Malcolm 1952; Stern and Wright 1956). These varieties, and others bred recently in Europe, are compared in yield trials at the Crop Research Division each season. The results in the present paper are from the only grain-quality tests made on the trial in 1956-57, and the only determinations of any sort made on samples from the trial of the previous season. Since 1957, malting-quality tests of samples from the variety trials at the Crop Research Division have been made in a pilot malting installation by the Canterbury (N.Z.) Malting Company, Ltd.

MATERIAL AND METHODS

Varieties

Results from six varieties are presented. The comments on quality given for the European varieties were obtained from the literature, particularly from NaCoBrouw yearbooks.

Carlsberg, a Danish variety (Nordgaarden) from Maja × Prentice. Very high yields, good quality. Carlsberg I, the original release, was sown in 1955; in 1956 a Danish re-selection, Carlsberg II, was also sown, and no sample of Carlsberg I was analysed. Carlsberg has given outstanding yields in New Zealand, and no differences between the two selections have been established.

Kenia, a relatively old Danish variety (Abed) from Binder × Gold. Good yields and quality, but replaced in Europe by better varieties. The standard variety in New Zealand for the heavier soils.

Maythorpe, an English variety (Cambridge Plant Breeding Institute) from Irish Goldthorpe × Maja. Good yields and very good quality, rather weak straw.

Piroline, a German variety (Heine) from Morgenrot × Weihenstephaner M.R.I. Very early, drought-resistant, good yields on lighter soils, fair quality.

Proctor, an English variety (Cambridge Plant Breeding Institute) from Kenia × Plumage Archer. Rather late, high yields in England and very good quality.

Research, reselected in New Zealand from the Australian variety of the same name (Werribee), from Plumage Archer × Prior. Good yields on lighter soils, weak straw, good quality.

Yield Trials

The trials at the Crop Research Division, Lincoln, were drilled in September of 1955 and 1956, with seed treated previously with hot water for the control of loose smut. There were ten replications, of seven-coulter plots two chains in length, sown in randomised blocks. Germination was satisfactory, and no effects from the hot-water treatment were visible. A composite sample of each variety was taken for quality tests.

In the 1955-56 season the weather was dry, with high temperatures. Growth of the barley was not vigorous, and there was no lodging. Proctor was the latest variety to ripen, and the trial was harvested with the header when Proctor was mature. The highest-yielding variety was Carlsberg I (63 bushels per acre), which outyielded Research, Kenia, and Proctor (56 bushels per acre), significantly. The differences between the latter three varieties were not significant. A sample of Maythorpe was obtained from an increase area sown adjacent to the trial on the same day.

In the following season conditions were more favourable, and there was slight lodging in the plots of Maythorpe and Research. The trial was headed in three stages, according to the times of ripening of the varieties. Piroline (93 bushels per acre), Carlsberg I, Carlsberg II and Research gave similar yields, significantly higher than Kenia and Maythorpe (73 bushels per acre). The yield of Proctor (63 bushels per acre) was significantly below that of Maythorpe. Proctor may have been affected by the hot-water treatment, as, in the following season,

when seed was not treated, Proctor yielded significantly more than Kenia and Research, though significantly less than Carlsberg.

Analytical Methods

Percentage of screenings. An *A6* screen was used. Subsequent analyses were carried out on the screened barley, and were expressed on a dry-matter basis.

Kernel weight. This was determined by weighing a sample of 1000 kernels.

Husk percentage. The method of Essery, Kirsop, and Pollock (1956) was used.

Nitrogen content. Total nitrogen of the grain was determined by the Kjeldahl method.

Percentage extract. The method of Pawlowski (1938, p. 43) was used.

Total and free β-amylase. These were determined by a modification of the method of Sandegren and Klang (Stern and Wright 1956).

RESULTS

The analytical results, expressed on a dry-matter basis, are presented in Table 1. Percentages of screenings are not included: eight of the determinations gave results between 5% and 10%, but in the first season Research and Proctor each had a 14% screening loss, and Kenia a 2% loss. No reason for the variation is apparent.

For each character, variety and seasonal differences were tested by the analysis of variance. The data for Piroline, which was grown only in 1956-57, were not included in the analyses, and Carlsberg I and II were treated as one variety. Only four degrees of freedom, measuring variety-by-trial interaction, were available to estimate an error mean square, but some of the differences between the trials, and the mean squares, have been examined in relation to independent estimates of variability obtained from analyses of manurial-trial data to be reported in the following paper.

Kernel Weight

No differences were significant. Research had the heaviest kernels in both seasons. Carlsberg, Kenia, and Maythorpe had heavier kernels in the first season than the second, Proctor and Research the reverse, showing a slight association of kernel weight with screening losses.

Husk Percentage

The difference between seasons was substantial, and almost significant at the 1% level, but variety differences were less marked, and not significant. Research had the highest percentage of husk in the first season and the lowest in the second, but differences between the other varieties were consistent: if the figures for Research were omitted from

TABLE 1. RESULTS OF BARLEY QUALITY TESTS

	Kernel weight g/1000	Husks %	Nitrogen %	Extract %	Total Amylase ‡	% Free Amylase
1955-56						
Carlsberg I	40.1	8.6	1.97	83.1	1001	61
Kenia	38.7	9.6	1.95	77.5	1054	54
Maythorpe	35.9	9.0	2.03	75.6	830	60
Proctor	35.1	8.8	1.98	76.3	1073	72
Research	41.6	9.7	2.00	76.6	996	70
Average	38.3	9.1	1.99	77.8	991	63
1956-57						
Carlsberg II	37.7	10.3	1.66	80.8	784	60
Kenia	35.2	12.4	2.06	78.9	665	22†
Maythorpe	33.7	11.8	1.79	75.3	866	52
Proctor	38.4	11.2	1.71	72.5	746	62
Research	43.2	10.1	1.65	76.9	691	72
Average	37.6	11.2	1.77	76.9	750	(54)
Piroline	38.8	11.6	1.62	79.1	821	56
S.D. (1)	2.1*	0.7	0.13*	1.5	119***	9**
S.D. (2)	1.1	0.6	0.07	1.1	24	3

(1) Standard deviation derived from analysis of above figures; 4 degrees of freedom.
(2) Standard deviation derived from manurial-trial data; 12 degrees of freedom.
* Significance of the difference between the standard deviations.
† Not accurate: this sample contained very little free amylase.
‡ Grammes maltose per 100 g barley dry matter.

the analysis it would be found that Kenia had a significantly higher husk content than Carlsberg.

Nitrogen Content

The difference between seasons was almost significant at the 5% level, and would be highly significant if tested against the manurial-trial variance. There was little variation between varieties in each season, except for Kenia in 1956-57, which gave the highest result of all.

Percentage Extract

There was little difference between the seasons, but the variety differences were considerable, and significant at the 5% level. The variety means are presented in Table 2, together with the grouping obtained from Duncan's test (Duncan 1955). Varieties with the same letter are not significantly different; for example, Kenia was not shown to be different from any other variety. The difference between Carlsberg and Proctor was highly significant, and Research and Maythorpe gave a significantly lower extract than Carlsberg.

TABLE 2. MEAN PERCENTAGE EXTRACT

	Mean % Extract	Group	
		5%	1%
Carlsberg	81.9	a	A
Kenia	78.2	abc	AB
Maythorpe	75.5	bc	AB
Proctor	74.4	c	B
Research	76.8	bc	AB
Piroline	79.1	ab	AB

Total β-amylase

Though there were varietal differences in each trial, they were not consistent. The seasonal difference, 241 units, was significant at the 5% level when tested against the variety-season interaction. This interaction was very highly significant, as shown in Table 1, but much of it may be explained by the relatively low amylase content of Maythorpe in the first season and of Kenia in the second, the first of these being associated with a fairly high nitrogen content and the second with a relatively high nitrogen content and a very low percentage of free amylase. Without these varieties the interaction would not be significant.

Percentage of Free Amylase

Neither variety nor season differences approached significance, but the variety-season interaction was highly significant. This interaction was produced by the aberrant behaviour of Kenia in the second season: if Kenia were excluded from the analysis the standard deviation (1) would be reduced to 4%, and the difference between Research and Maythorpe would be significant at the 5% level. The low value for Kenia is much lower than any result obtained in the previous study (Stern and Wright 1956). There, the lowest free amylase value was 31%, obtained, like the present one, from a sample of Kenia with a high nitrogen content and a low total amylase content.

Ratio of Amylase to Nitrogen

This ratio was found useful in comparing samples of Kenia and Research (Stern and Wright 1956). In the present data, as would be expected from the above discussion, the ratios for Kenia and Maythorpe were not consistent. No differences among the other varieties approached significance, but the highest ratio in the second season was given by Piroline.

DISCUSSION

The differences found between seasons in the present data were in agreement with those in the earlier extensive study of Research and Kenia samples; in the drier season nitrogen and total amylase contents, and also the ratio of amylase to nitrogen, were higher.

While it is obvious that the present results do not provide accurate assessments of the quality characteristics of the barley varieties, they have given useful information. It is to be expected that the varieties will differ in all characters, though the only character for which any reliance could be placed on the present variety averages is percentage extract. For kernel weight, nitrogen content, total β-amylase and percentage of free amylase it has been suggested that there are real variety differences, which vary from season to season, or at least from trial to trial, and because of this variation much more extensive data would be needed before the varieties could be placed with any confidence in an order of merit. It is unlikely, however, that any of the European varieties studied will prove to be significantly better than Research in husk percentage, nitrogen content or percentage of free amylase.

The values found for extract may be compared with those obtained from trial samples in England (Elliott and Burrell 1956). As summarised in that report, on the basis of small-scale malting tests, Maythorpe gave a considerably higher extract than Proctor. Carlsberg and Kenia were lower in extract than Proctor, but were not compared directly with each other. Pilot malting tests on the 1956-57 samples at the Canterbury (N.Z.) Malting Company, Ltd., have given results similar to these, and Research and Piroline gave lower extracts than Kenia. The lack of agreement with the present results, at least for Piroline, Maythorpe, and Proctor, is substantial, and is presumably due to the nature of the tests, which were made on barley (this paper) and on malt (Malting Company). It is probable that the tests on malt are in general the more valuable, but with a variety of which the malting behaviour is not thoroughly known—and this means, for New Zealand, all but Research and Kenia—the malting tests involve some uncertainty as to optimal treatment, and the results which have been obtained cannot give a final answer.

New brewing processes, incorporating wort storage and continuous fermentation, are being introduced commercially by New Zealand brewers, and new types of malt are required for them. There is at present a place for Research, and for Kenia, which, compared with Research, gives a higher extract of malt with a lower modification capacity, and there may also be a place for some of the other varieties, particularly Carlsberg and Maythorpe. The outstanding characters of these other varieties, grown in New Zealand, as determined in the case of quality factors from only two samples grown at Lincoln, are as follows:

Carlsberg: high yield, low husk content, high extract in barley test.

Maythorpe: low free-amylase content, high extract in malting test.

Piroline: early-maturing, low nitrogen content, high amylase/nitrogen ratio.

Proctor: late-maturing, possibly low yield, low extract in barley test.

REFERENCES

Duncan, D. B. 1955: Multiple Range and Multiple *F* Tests. *Biometrics 11:* 1-42.

Elliott, C. S.; Burrell, W. D. 1956: Spring Barley Variety Trials, 1948-53. *J. nat. Inst. agric. Bot. 7:* 551-64.

Essery, R. E.; Kirsop, B. H.; Pollock, J. R. A. 1956: Studies in Barley and Malt. V. Determination of Husk-content and Mealiness of Barley. *J. Inst. Brew. 62:* 150-2.

Malcolm, J. P. 1952: "Varieties of Malting Barley." Bull. No. 2, The Canterbury (N.Z.) Malting Co. Ltd., Christchurch.

Pawlowski, F. 1938: "Die Brautechnischen Untersuchungs-Methoden." 5th ed. Oldenbourg, Munich and Berlin.

Stern, R.; Wright, G. M. 1956: Nitrogen and Amylase in Some New Zealand Barleys. *J. Inst. Brew. 62:* 470-7.

STUDIES ON SEPTORIA LEAF BLOTCH DISEASE OF WHEAT (*Triticum aestivum* L.) CAUSED BY *Septoria tritici* Desm.*

By H. T. Wenham, Massey Agricultural College, Palmerston North

(Received for publication, 1 September 1958)

Summary

Septoria tritici Desm. is a common foliage pathogen of wheat in Canterbury.

Symptoms are brown to straw-coloured lesions which may extend across leaves causing death of tissues towards the tip. Pycnidia are produced abundantly and are confined to areas between the veins.

Morphology, spore germination and cultural features of the causal fungus are described.

Nomenclature of the pathogen is discussed.

The disease, which in New Zealand is confined to wheat, is perpetuated by infected volunteer wheat plants and by pycnidia in crop debris.

Introduction

A septoria foliage disease of wheat was first recorded in New Zealand by Cunningham (1927) who cited *S. graminum* Desm. as the causal fungus. Prior to 1947 the disease had been considered of minor importance in New Zealand, but in November of that year a number of crops in the vicinity of Lincoln were observed to have a high percentage infection. In a survey of 40 wheat crops in north Canterbury conducted in November 1948, only two crops were free from infection. In three crops 70% of plants were estimated to be diseased at that time. The disease was recorded as present in 43 of 56 crops inspected in Canterbury during the spring of 1953 (Anon. 1953). Septoria leaf blotch has since been prevalent each year, particularly during periods of prolonged cool wet weather.

The disease is world-wide in distribution and of considerable economic importance in many countries (Luthra *et al.* 1937, Fomin 1935, Boewe 1939, Sprague 1944). In view of its potential importance as indicated by overseas reports, a study was undertaken of the disease and its causal organism under New Zealand conditions.

Symptoms

The disease is expressed as lesions on both upper and lower leaf surfaces, such areas being covered with golden-brown to black pycnidia (Fig. 1). On susceptible varieties sown in autumn in Canterbury, symptoms are in evidence soon after seedlings are above ground as isolated brown blotches approximately 5 mm in width which enlarge,

* This paper is abstracted from a thesis presented to the University of New Zealand in partial fulfilment of the requirements for the Degree of Master of Science.

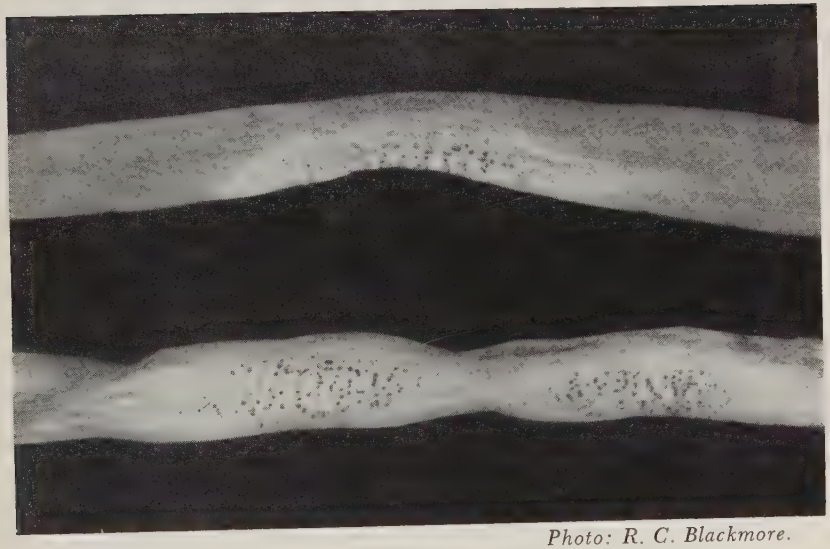


Photo: R. C. Blackmore.

Fig. 1.—Wheat leaves infected with septoria leaf blotch, showing pycnidia of the causal fungus.

coalesce, and frequently extend across the leaf, causing death of all tissues towards the tip. Pycnidia are usually abundant, are confined to areas between the parallel veins, and stand out prominently against the dead host tissues. In cases of severe infection all lower leaves are killed and contain large numbers of pycnidia, the density of which is a striking feature of the disease at this stage. During winter the disease develops little but on new spring growth further distinct yellow-brown lesions appear, which usually remain as small rectangular areas extending toward the tip. The pycnidia, then not produced in such great numbers, are light brown, and this together with the small total leaf area affected combines to make the disease relatively inconspicuous at this stage.

MORPHOLOGY

Pycnidia were usually flattened and ellipsoid, deeply embedded in host tissue and ranged in diameter from 50 to 190 μ (av. 118.6 μ). Pycnidia walls were thick, well defined and consisted of coarse densely interwoven hyphae (Fig. 3). Pycnidiospores (Fig. 4) were filiform, hyaline with rounded ends, usually 3 septate, and 20 to 64 μ by 1.75 to 2.1 μ (av. 42.71 by 1.95 μ). Spore germination in water and on P.D.A. was slow and was initiated by elongation of the spore for approximately 12 hours followed by development of one or more germ tubes, usually at 45° to the longitudinal axis of the spore (Fig. 5).

TAXONOMY

Septoria tritici was first described by Desmazieres (1842) on wheat specimens he received from Roberge. The following year he presented a meagre description of *Septoria graminum* on leaves of an unidentified grass, commenting that it was similar to *S. tritici* (Desmazieres 1843).

Photo: R. C. Blackmore.

Fig. 2.—Culture of *S. tritici* on P.D.A. held at 24°C for 19 days.

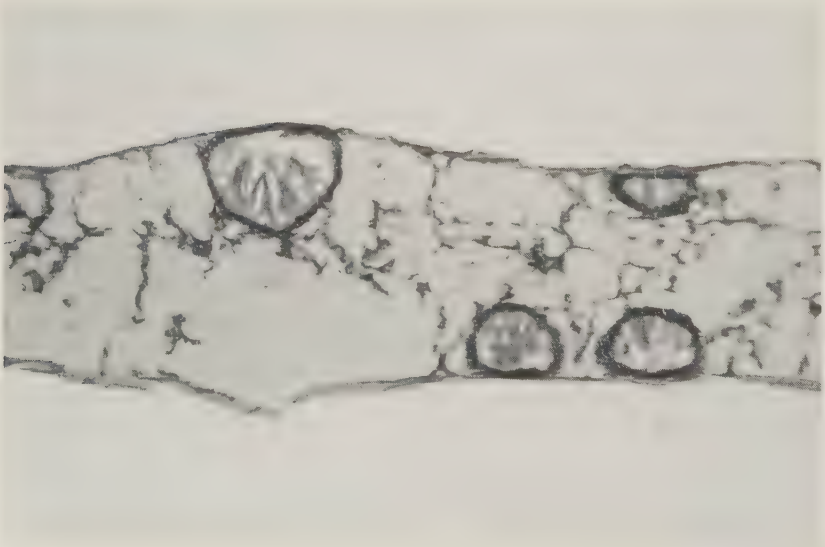


Photo: R. C. Blackmore.

Fig. 3.—Longitudinal section of infected leaf showing pycnidia.

Fig. 4.—Pycnidiospores from field-infected Cross 7 wheat.

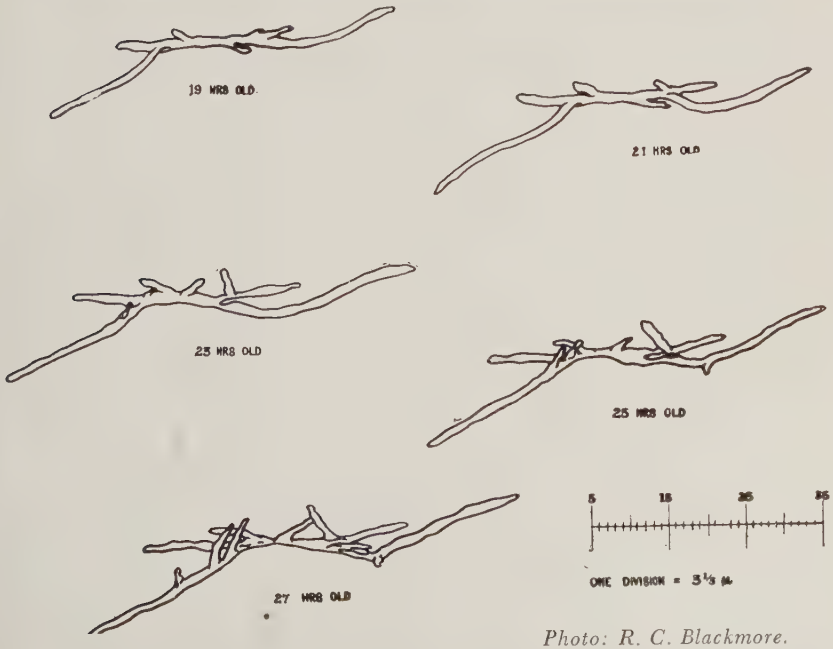


Photo: R. C. Blackmore.

Fig. 5.—Latter stages in germination of a pycnidiospore on P.D.A.

Later he reduced *S. tritici* to varietal rank under *Septoria graminum* (Desmazieres 1847), and this terminology has since been widely adopted, in many cases only the species name (*Septoria graminum*) being applied to the fungus on wheat. Cunningham (1927) apparently followed this practice when referring to the disease in New Zealand. Sprague (1938) reviewed the status of *S. graminum* Desm. and showed it to be confined to *Brachypodium* spp. and quite distinct from *S. tritici* Desm. on wheat. It is therefore recommended that the name *S. graminum* Desm. given by Cunningham to the fungus attacking wheat in New Zealand be replaced by *S. tritici* Desm. This would conform with the revised nomenclature overseas.

The other septoria disease of wheat, caused by *S. nodorum* Berk. has not been recorded in New Zealand.

Host Range

Sprague (1944) records *Septoria tritici* as pathogenic to the following graminaceous species: *Lolium perenne, L. multiflorum, Holcus lanatus, Poa pratensis, P. annua,* and *Avena sativa.* In no case have they been recorded as alternate hosts to the disease in New Zealand. To determine whether the pathogen could survive on any of these species all were grown in pots of steam-sterilised soil and artificially inoculated with a spore suspension prepared from actively growing colonies isolated from wheat. All plants remained disease free, whereas wheat plants sprayed with the same spore suspension developed typical symptoms.

Crops of Russet and Algerian oats sown in February 1949, in stubble of wheat crops severely diseased the previous year were closely inspected for the disease. Whereas volunteer wheat plants were heavily attacked, both oat varieties remained free from infection.

It is concluded that in New Zealand *S. tritici* is highly specialised and is confined to wheat.

Perpetuation of the Disease

In Canterbury most wheat crops are sown in autumn and harvested by February. The causal fungus must therefore survive up to four months (February to May) to provide new primary inoculum. Volunteer wheat, alternate hosts among the Gramineae, and pycnidia in wheat debris have all been reported overseas as means whereby the disease may be perpetuated (Weber 1922, Boewe 1939, Chester 1947). In view of the uncertainty of appearance of volunteer wheat and the absence of alternate hosts in New Zealand, the importance of crop debris as a means of survival was investigated.

Threshed wheat straw is commonly held in stacks for some months. Beginning in February, leaves with pycnidia of the causal fungus were collected at 21-day intervals and hanging drop slides prepared of pycnidiospores. The slides were incubated at 22°c and the germination capacity of the spores noted. It was found that such spores remained viable for at least five months. During June the percentage of spores germinating declined rapidly and by July only an occasional spore showed signs of germination.

Infected volunteer wheat plants have been located each month of the year and thus may serve as foci of infection from which the disease could spread to sown crops. Results of the above experiment indicate that the fungus may also persist through the period February to May as viable spores within pycnidia of diseased leaves. With leaf decay, fragments bearing pycnidia may be dispersed by wind and serve as a further source of primary inoculum.

CONTROL

The most practical approach to control of septoria leaf blotch is through host resistance, but the disease is at present not considered of sufficient importance to warrant a breeding programme aimed specifically at producing resistant varieties. The variety Cross 7 is highly susceptible, but the newer varieties Hilgendorf, Aotea, and Arawa, all show considerable field resistance and the importance of the disease is decreasing as Cross 7 is being grown on a smaller area. Practices which will limit sources of primary inoculum would be beneficial; thus autumn volunteer wheat should be removed and straw and stubble of infected crops be destroyed either by deep ploughing or burning. Besides destroying pycnidia of *S. tritici*, this latter practice would also reduce eyespot (*Cercosporella herpotrichoides* Fron.) and powdery mildew (*Erysiphe graminis* DC.), both of which are known to be perpetuated by way of crop debris.

ACKNOWLEDGEMENT

The author wishes to record his indebtedness to Dr. I. D. Blair, of Canterbury Agricultural College, under whose direction this investigation was carried out.

REFERENCES

Anon. 1953: *New Zealand Wheat Review; 1950, 1951, 1952.* p. 21.
Boewe, G. H. 1939: *Ill. nat. Hist. Surv. Circ. 35.*
Chester, K. S. 1947: "Nature and Prevention of Plant Diseases". H. K. Lewis, London.
Cunningham, G. H. 1927: *Int. Bull. Pl. Prot. 1:* 157-8.
Desmazieres, J. B. H. J. 1842: *Ann. Sci. nat. bot., Ser 2, 17:* 91-107.
————1843: *Ibid., Ser. 2, 19:* 335-73.
————1847: *Ibid., Ser. 3, 8:* 9-37.
Fomin, E. 1935: *Bull. Ukr. sci. Res. Inst. Grain Cult. 1:* 88-133.
Luthra, J. C.; Sattar, A.; Ghani, M. A. 1937: *Indian J. agric. Sci. 7:* 271-89.
Sprague, R. 1938: *Mycologia 30:* 672-8.
————1944: *Ore. St. Monogr. Bot. 6.*
Weber, G. F. 1922: *Phytopathology 12:* 517-85.

NEW ZEALAND 48s COUNT ROMNEY CROSSBRED WOOL

By D. A. Ross, New Zealand Wool Industries Research Institute, Dunedin.

(*Received for publication, 15 September 1958*)

Summary

An investigation was carried out into the characteristics of 87 samples graded by New Zealand woolclassers as 48s count Romney Crossbred wool. Mean values for the main characteristics measured were: grease 5.1%, suint 7.6%, yield at 16% regain 79.5%, fibre diameter 37.5 μ, crimps per inch 3.2, staple length 6.9 in., medullated fibres 6.1%. Measurements of fibre length and staple length were also made.

The samples were regraded by 10 senior woolclassers and these regradings and a suggested new grading nomenclature system are discussed.

Introduction

In recent years there has been an increasing awareness of the necessity for determining the importance of the physical characteristics of a wool such as crimp, length, handle, and diameter, during the manufacturing process and in the final cloth. Such knowledge is essential for any research project which has as its ultimate aim the production of wools with the most desirable manufacturing properties. Research on this problem has commenced at the Textile Research Institute at Princeton, U.S.A., where initial investigations have been aimed at assessing the part played by crimp during manufacture. Two batches of Merino wool with different numbers of crimps per inch but with similar mean fibre diameter have been processed, and their manufacturing performance and the characteristics of the final cloths compared. It was during a discussion on the possibilities of carrying out a similar type of trial with New Zealand Crossbred wools that it was brought forcibly to our notice that published data on the characteristics of New Zealand Romney Crossbred wool were practically non-existent.

Henderson and McMahon (1947) found that 74% of the total greasy clip of the 1943-44 season was wool of counts 46s to 50s. This would be largely wool from Romney Crossbred sheep. Until we have adequate data on the characteristics and uses of this group of wools which are the main types we grow, it is quite impossible to plan a comprehensive programme for improving New Zealand's wool. The principal aim of this investigation therefore was to obtain some data on one of our main wool types, namely 48s count Romney Crossbred wool.

Because of the many meanings of the term quality in the wool trade, the term count has been used in preference to quality or quality number.

The use of synthetic fibres has highlighted the very definite advantages during manufacture of processing fibres which conform to

known physical and chemical standards. The manufacturing performance of such fibres of known specifications can be fairly accurately predicted. Much of this advantage is due to the dimensional uniformity of synthetic fibres, a uniformity which cannot be achieved with wool by reason of its very nature. However, similar data for wool would help to reduce the guessing element associated with the wool trade which is not in the best interests of the wool trade as a whole and therefore is detrimental to New Zealand wool growers.

Materials and Methods

Two trials on 48s count wool were run, designated Trial A and Trial B. Trial A samples were obtained during the 1955-6 wool season and Trial B during 1956-7.

Source of Samples

Trial A. Sixty-six samples were tested, 44 selected by classers at 36 woolstores throughout New Zealand, 20 selected by New Zealand Wool Commission appraisers at stores representing all the different selling centres in New Zealand, one from a Department of Agriculture Sheep and Wool Instructor and one from an Agricultural College.

Trial B. Seventeen samples were tested, 15 from different woolstores and 2 from Agricultural Colleges.

Specifications for Samples

Trial A. "One large handful to be taken from the side of a single, full year's growth, Romney Crossbred ewe fleece. The wool should be a sound combing wool, and should have what you consider to be a typical crimp and fibre fineness for 48s count."

Trial B. "From quarter to half a pound of wool from the side of a single fleece of a Romney Crossbred ewe. The wool should be AA or A style as classified on the Bareme scale, and have what you consider as near perfect as possible crimp and fibre fineness for a 48s count wool."

Testing Procedure

All tests which are affected by atmospheric conditions were carried out in a conditioning room maintained at 68°F and 65% r.h.

Subsamples of 10–20 g were withdrawn and tested for grease and suint content, and yield, as follows.

Grease. The greasy wool samples were conditioned for at least 24 hours before weighing. The grease content was determined by extraction with petroleum ether (b.p. 40–70°C) using a Soxhlet apparatus. The extract was filtered through cotton wool before evaporating to dryness and weighing.

Suint. Following degreasing, the wool was washed 7 times with distilled water to remove the suint. The combined extracts were centrifuged to remove the dirt, and aliquots were evaporated to dryness and weighed.

Yield. After removing the grease and suint the samples were oven dried to constant weight at 105°c. From this weight the yield of clean wool at 16% regain was calculated. No separate determination of dirt and vegetable matter was made, as the samples were all free from any obvious vegetable matter and dirt.

Fibre Diameter. Each of the extracted wool samples used for yield determinations was conditioned and thoroughly mixed. A subsample was placed in a Hardy microtome and a section 0.8 mm cut for fibre diameter measurements on a projection microscope at a magnification of 500. Two observers each measured 250 fibres on each sample, from which the length-biased mean diameter was calculated. A rapid measurement of the fibre diameter of these samples was also obtained by using the Airflow apparatus.

Medullated Fibres. During the measurement of the diameter of the fibres on the projection microscope the number of medullated fibres in each sample was noted. No record was made of varying degrees of medullation.

Staple Length. Ten staples were drawn from different parts of the sample and measured for length without stretching the staples.

Fibre Length. Fibre length measurements were made on fibres from 11 samples which had staple lengths close to the group average. The staples were first measured for length then a group 2–300 fibres was dissected from the side of the staple. The lengths of all the fibres in the subsample were measured on a Wool Industries Research Association Fibre Length Machine, which straightens the fibres under a small tension and places them in length groups differing by 0.5 cm.

Crimps per Inch. While the measurement of crimp is relatively easy in fine-woolled sheep like the Merino and Corriedale it is much more difficult in strong-woolled sheep where there are frequently marked changes in crimp along individual staples. The use of a "crimp wheel" was not satisfactory for these wools and a method was used in which the staples were placed on a sheet of paper on top of a "light-box" and the paper marked at the peak of each crimp. From the average spacing of the marks the number of crimps per inch was calculated.

Staple Strength. The major fault in New Zealand wools is the very high percentage of fleeces which show break or tenderness. Tender wool breaks easily during processing, resulting in a higher noil production and a weaker yarn than is obtained by processing sound wool.

Although the sample specifications were for sound wools, some of the samples received were very tender. It was therefore decided to test the staple strength of the samples.

Full details of the method developed for measuring the strength of the staples have yet to be published. In brief, the butt $\frac{3}{4}$ inch of staples to be tested was clamped in a pair of leather-lined clamps. A second pair of clamps was placed $2\frac{1}{2}$ inches higher up the staple. The staple was then broken on an autographic yarn-strength tester which had a constant rate of traverse of the bottom jaw of 4 in/minute. After

breaking, the wool projecting from the clamps was cut off, washed successively in petroleum ether and warm water, dried to a low regain and then conditioned at 68°F and 65% r.h. for at least 24 hours before weighing. The work to break was then calculated as cm lb/g and the breaking load as lb/g at a breaking length of 2½ in. Two staples per sample were tested in Trial A while, in Trial B, 5 staples per sample were tested for breaking load only.

Results

The mean values, standard deviations, and ranges of values for each of the characteristics measured in Trials A and B are given in Table 1. The mean values for all samples are also given and as there was little difference between results found for the two trials, for most purposes the results can be considered as a single group.

TABLE 1. Characteristics of New Zealand 48s Count Romney Crossbred Wool

	Trial A 66 Samples			Trial B 17 samples			Trials A and B 83 samples	
	Mean	S.D.[1]	Range	Mean	S.D.	Range	Mean	S.D.
Grease, % on C.G.W.[2]	5.2	1.4	1.6– 8.5	4.6	1.1	2.9– 6.6	5.1	1.3
Grease, % on C.O.D.W.[3]	7.7	2.2	2.0– 13.1	6.6	1.8	4.0– 10.1	7.5	2.2
Suint, % on C.G.W.	8.0	2.0	2.2– 12.1	5.8	1.7	2.6– 9.2	7.6	2.1
Suint, % on C.O.D.W.	12.0	3.4	2.7– 19.5	8.4	2.8	3.4– 13.9	11.2	3.6
Yield, % at 16% regain	78.9	4.5	71.3– 93.5	81.5	4.4	73.4– 90.2	79.5	4.6
Fibre diameter, μ	37.3	2.5	29.9– 42.1	38.3	2.1	35.4– 42.8	37.5	2.4
Fibre diameter, S.D. μ	8.5	1.0	6.7– 11.3	8.3	1.2	6.6– 11.6	8.5	1.1
Fibre diameter, C. of V. %[4]	22.9	2.9	18.4– 30.2	21.6	2.9	16.6– 28.3	22.6	2.9
Fibre diameter, Range μ	46.5	5.5	60 – 34	45.5	5.0	54 – 38	46.3	3.0
Medullated fibres, %	5.8	7.1	0.0– 39.3	7.6	9.7	0.9 - 37.5	6.1	7.6
Crimps per inch	3.2	0.5	1.9– 4.3	3.2	0.5	2.6– 4.6	3.2	0.5
Staple length, in.	6.8	0.9	5.2– 10.3	7.2	0.7	6.1– 8.3	6.9	0.9
Work to break, cm lb/g (2½ in.)	427	187	93 –966	—	—	— —	—	—
Breaking load, lb/g (2½ in.)	94	46	14 –228	142	34	99 –196	104	46

[1] Standard Deviation
[2] Conditioned Greasy Weight
[3] Clean Oven-dry Weight
[4] Co-efficient of Variation %

The only notable difference between the two groups was the better style of the samples in Trial B. These samples had a mean grading closer to A style than to BB style, while the Trial A samples had a mean grading about midway between BB and B style. While all the Trial B samples were sound, quite a number of the Trial A samples had severe lambing breaks or were otherwise tender.

Grease, Suint, and Yield

Compared with Merino wools, the average grease content of these Crossbred samples (5%) is low. However, it still means that about 10,000 tons of wool grease are produced annually in New Zealand of which only a small amount is recovered, either here or overseas. An even greater weight of suint also goes down the drains each year and will continue to do so until uses which make suint recovery commercially profitable are found.

The mean yield of 79.5% at 16% regain is high for Crossbred wool but in line with yields reported by Henderson and McMahon (1947) for A or BB style wools. There are several reasons for the high average yield: the wools sent in were all clean side wools; secondly, soxhlet extraction results in a slightly higher estimate of yield than hand scouring; thirdly, included among the samples were a number of rain-washed wools from the very high rainfall area of Westland, which yielded as high as 93.5%.

On the basis of this analysis, 100 lb of conditioned greasy N.Z. 48s Romney Crossbred side wool would contain:

	lb
Clean scoured wool at 16% regain	79.5
Grease	5.1
Suint	7.6
Suint moisture and dirt	7.8
Conditioned greasy wool	100.0

Therefore for every 100 lb of clean oven-dry wool there would be

	lb
Grease	7.4
Suint	11.1
Suint moisture and dirt	11.4
Regain moisture	16.0
	45.9

TABLE 2. CORRELATIONS BETWEEN FLEECE CONSTITUENTS

	Grease %	Suint %	Moisture %
Suint %	0.200	—	0.437*
Yield %	0.472*	— 0.827*	— 0.771*

* $P < 0.01$

Thus even in high yielding wool like these samples of 48s there is about half a pound of non-wool material for every pound of dry wool substance.

The correlations between some of the fleece constituents are given in Table 2 for all samples.

The moisture figures used in Table 2 are 100 − (grease % + suint % + oven-dry wool %), no allowance being made for dirt, while yield is at 16% regain. With the exception of the correlation between grease and suint which does not quite reach the 5% level of significance all the correlations are highly significant at the 1% level.

It is easier to determine the moisture or suint content than it is to measure yield. However, the standard errors of the regressions for predicting yield from the suint or moisture content were found for all samples to be 2.60% and 2.94% respectively. These are rather high even for a rapid technique for yield determination.

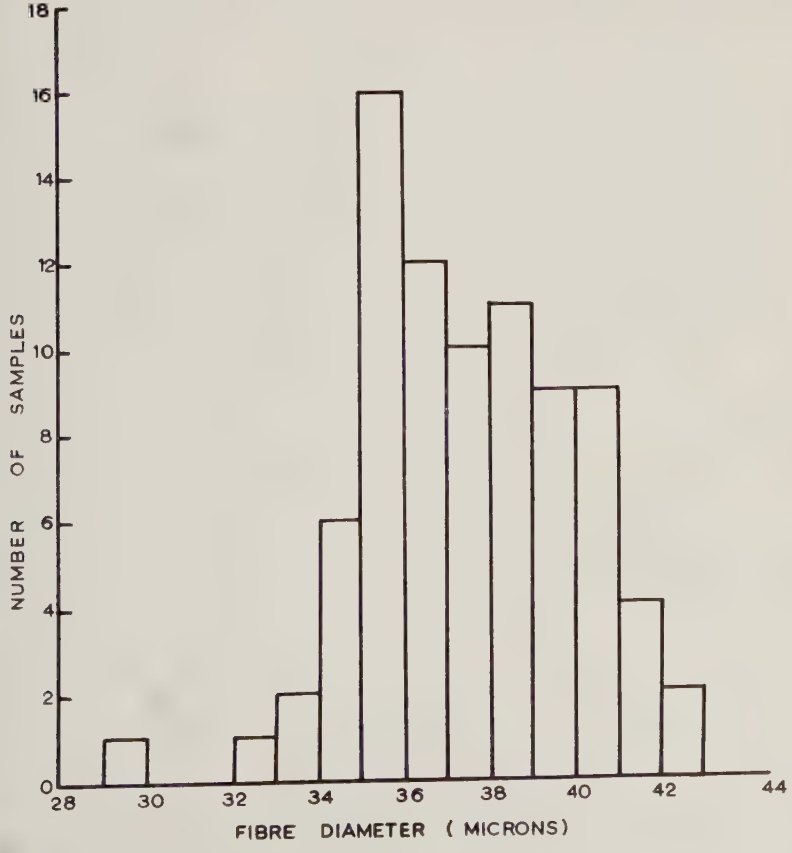


Fig. 1.—Distribution of mean fibre diameter of samples.

Fibre Diameter

Projection Microscope. The most important of the physical characteristics of a wool is its fibre diameter. It is the fibre diameter which largely determines the use and the performance of a wool during manufacture.

The mean diameter of all the samples from both Trials A and B of 37.5 μ (Table 1) was higher than expected on the basis of top diameters reviewed by Hindson and Young (1955). From the distribution of sample diameters as shown in Fig. 1, it may be seen that only 10 of the 83 samples were finer than 35 μ while 35 were coarser than 38 μ, 36 being between 35 and 37 μ. The better style samples of Trial B were as coarse as those of Trial A with similar mean and within-sample variation in fibre diameter. It would appear doubtful therefore if improving the style of wool will reduce within-sample fibre diameter variability. The mean range of fibres within samples (Trial A, 46.5 μ; Trial B, 45.5 μ) are also very similar as would be expected from the similar mean standard deviations.

The finest sample, which had a mean diameter of 29.9 μ, was no doubt classed as a 48s because of its coarse crimp, 2.7/inch, and its harshness of handle.

With increasing fibre diameter the increase in the standard deviation for samples in both Trial A and Trial B is small; $r = 0.209$, which is just below the 5% level of significance. The variation as expressed by the coefficient of variation decreases with increasing fibre diameter ($r = -0.307$; $P < 0.01$) because the small increase in standard deviation is associated with a larger increase in mean diameter resulting in a smaller coefficient of variation.

Airflow Fibre Diameter. A detailed account of the methods and results obtained when comparing airflow and projection-microscope measurements of diameter of the Trial A samples has been given elsewhere (Ross 1958). The standard error (0.77 μ) of the airflow estimate as compared with the projection microscope mean diameter was very low, showing that the airflow apparatus is suitable for obtaining a rapid measurement of the fibre diameter of Crossbred wool. The height of the float scale on the airflow apparatus was corrected after the Trial A samples had been measured and the Trial B samples were measured by the same method approximately a year later. The difference of the means of the 17 samples as measured by the two methods was 0.03 μ while the standard error of the airflow estimate was 0.99 μ.

Medullated Fibres

The percentage of medullated fibres is only a poor guide to the amount of medulla by weight or volume in a wool sample. A few isolated specks of medulla in a high percentage of fibres will neither be apparent to the eye nor noticeably affect the dyeing or manufacturing performance of a wool, on the other hand a small percentage of heavily medullated fibres will be apparent both in greasy wool and after dyeing.

As shown in Table 1, the percentage of medullated fibres was higher in Trial B than in Trial A, but the standard deviations, which are higher than the means, indicate that the populations did not have normal distributions. Omitting 11 samples which had more than 14.9% of medullated fibres from the total lowers the mean value of the remaining 72 samples to 3.7% of medullated fibres.

In those samples which had a high percentage of medullated fibres the medulla was obvious as a hairy tip. The sample in Trial B with 37.5% of medullated fibres appeared to be a good A style wool with a strong tip. When examined under benzene, however, the tip half and the butt quarter of the staple showed many very heavily medullated fibres.

Crimp

The method described for the measurement of crimp appears to be quite satisfactory for Crossbred wool.

The coefficients of variation between staples within samples were 10.6% and 11.5% for Trials A and B respectively. The better style Trial B samples were therefore as variable in the number of crimps per inch within samples as those of Trial A.

That crimp is important in assessing count is shown by the same mean value of 3.2 crimps per inch for both trials A and B. The range of from 1.9 to 4.6 is however rather wide. Considering the whole range of quality numbers there is no doubt that the number of crimps per inch increases with increasing fineness. Within any one count group, however, there is such a wide range of crimps per inch that count gradings based on crimps per inch must of necessity be rather variable in fibre diameter. The correlation between mean fibre diameter and number of crimps per inch for all samples was not high ($r = -0.277$; $P < 0.02$) but shows as expected that with increasing diameter there is a decrease in the number of crimps per inch.

Staple Length

With the exception of one sample, mean staple lengths varied from 5.2 to 8.6 inches with a mean of 6.9 inches (17.2 cm).

Even the shorter samples were long enough to be Noble combed while some might be preparing types rather than combing wools.

The C. of V.% between staples within samples for the Trial B wools was only 5.4%, and fewer staples could have been measured per sample with little loss in accuracy.

Fibre Length

Pohle *et al.* 1953 found with fine wool that the mean fibre length in the top was similar to the staple length. The mean fibre length before processing would therefore be expected to be longer than the staple length because of breakage of fibres during processing.

An average of 284 fibres was measured on each of 11 samples. The results, which may be low because of some fibre breakage during measurement, are shown in Table 3.

TABLE 3. Fibre Length Measurements

Staple length, mean, cm	17.2
Staple length, range of sample means, cm	15.7–18.3
Fibre length, mean, cm	18.2
Fibre length, range of sample means, cm	16.4–19.5
Fibre length, mean standard deviation, cm	5.6
Fibre length, mean coeff. of variation, %	30.7

Staple Strength

It can be seen from the work and breaking load results given in Table 1 that some of the samples of Trial A had severe lambing breaks. These samples therefore did not conform to the sample specifications of sound combing wools.

The correlation between work and breaking load for the Trial A samples was very high, r = 0.930, and because of this and the fact that it is much simpler to measure breaking load than work, only breaking load was measured on the Trial B samples.

One of the major differences between the results of Trials A and B was the difference in staple strengths. Even the weakest of the Trial B wools which had a breaking load of 99 lb/g was stronger than the mean of the Trial A series of 94 lb/g. The Trial B samples would be expected by specification to be stronger.

The 14 samples which broke below 50 lb/g should not be classified as sound combing wools while the 25 from 50 to 100 lb/g are not completely sound. There was a general reduction in staple strength associated with the time of shearing of the wools sampled, those wools shorn early in the season being stronger than those shorn later.

The low breaking loads of some of the samples measured for fibre length may have resulted in some fibre breakage during measurement resulting in a low value for mean fibre length.

Regrading of Trial A Samples

When all the Trial A samples were viewed together it was obvious that they actually covered a range of counts, and ten senior woolclassers were individually asked to count grade the samples, using half-grades, e.g. 46/48s where desired. Each classer's grading was scored according to a numerical index and from these figures an average was calculated for each sample. As the classers knew that they were regrading samples sent in as 48s this may have tended to affect their average grading.

On the numerical scale used (. . . 46s = 5, 46/48s = 6, 48s = 7, . . . etc.) the lowest classer had a mean for all samples of 6.1, equivalent to 46/48s, and the highest 7.8, equivalent to 48/50s, while the mean of all gradings was 7.0 (48s exactly). Two classers graded the samples over a range of 5 counts (10 units), e.g. 44s–52s while the

average range of the 10 classers was just below 4 counts (approx. 7 units) e.g. about 44/46s to 50s.

The count distribution of the samples after regrading is given in Table 4 together with the mean values for various characteristics of the samples.

TABLE 4. Mean Sample Measurements of Regraded Groups

Mean count	44/46s	46s	46/48s	48s	48/50s	50s
No. of samples	1	2	15	28	15	5
Crimp/inch	1.8	2.3	2.8	3.2	3.5	4.0
Diameter, μ	39.7	38.4	38.5	37.1	36.3	36.0
Mean S.D., μ	—	—	8.6	8.5	8.3	8.0
Mean C. of V., %	—	—	22.6	23.0	22.8	22.4
Staple length, in	—	—	7.1	6.8	6.6	6.5
Medullated fibres, %	—	—	8.1	5.9	4.0	4.8
Yield, %	—	—	81.4	77.9	78.9	75.4
Grease, %	—	—	4.0	5.4	6.1	5.5
Suint, %	—	—	7.3	8.7	7.6	8.1

Of the 66 samples, 58 had an average regrading of 46/48s, 48s, or 48/50s; five of the remainder were graded as 50s, two as 46s, and one as 44/46s. It may be seen that there is a very good relationship between mean diameter, crimp, and count, although the diameters of the 28 samples regraded as 48s actually ranged from 32.5 to 42.1 μ. The mean diameter decreases with increasing count number while the number of crimps per inch increases. Even within the narrow range of counts of 46/48s, 48s, and 48/50s, the classers have consciously or subconsciously graded for differences in diameter and number of crimps per inch. Their accuracy is good as regards the mean of a number of samples, but individual samples may vary widely in diameter or crimp.

The mean values for standard deviation of fibre diameter, staple length, percentage of medullated fibres, and yield are also in the expected relationship to count, decreasing with increasing count numbers. The grease content increases with increasing count number while the suint content is variable.

TABLE 5. Count Ranges on Regrading

Count Range	Number of Samples
0	1
½	2
1	31
1½	13
2	18
3	1

The range of counts, i.e. the difference between the highest and the lowest value which was given to each of the samples by the wool classers, is shown in Table 5.

Only 1 sample (sent in by an Agricultural College) was given the same count (48s) by all the classers, 31 samples covered a range of one count difference, while 18 covered a range of two counts, e.g. 46s to 50s, or 48s to 52s; 1 sample was graded over a 3-count range from 40s to 48s.

These gradings again show the fallibility of eye judgement and the difference of opinion between experienced classers as to the count of individual samples.

General Discussion

As would be expected, there was considerable variation between the measured characteristics of the samples sent in as of 48s count. There were differences between samples from different centres but as the number of samples from any one centre was small these differences are of doubtful significance.

These samples represent only a minute fraction of all the 48s count wool produced annually in New Zealand, and, as such, care must be taken in generalising about 48s from these results. They do, however, provide a basis for replacing some ideas and impressions about this grade of wool with some facts. We now have a firmer foundation on which to base the selection of wool of this count for manufacturing trials.

The high percentage of suint in the wools together with the 5% of grease emphasises the need for further research to be undertaken to make these products a profitable instead of a low-valued but unavoidable by-product of wool production.

The major factor which governs the uses and the performance of a wool during manufacture is its fibre diameter. Other characteristics such as length, colour, handle, yield, etc., affect the assessment of commercial value but only within a limited price range set by the buyers assessment of the fineness of the wool. Such an estimate of fineness is not made in microns but in terms of count with reference to a type of wool, e.g. Romney Crossbred or Corriedale.

Initially count was related to the spinning value of a wool, but today, with few exceptions, wools are no longer spun to their count numbers nor in most cases can they be on modern machinery. Therefore count number is now a subjective grading and there is considerable variation in its estimation both between individual wool classers and classers of different countries and also because of changes in the standards of individual classers.

Some of the more important characteristics affecting the assessment of count of Crossbred wool are the degree of crimping, staple length and the type of tip of the sample.

Over the years there has been much controversy about the relationship between count and fibre diameter and the value of diameter

measurements in trade practice. There is no disputing the fact that as count decreases diameter increases. Samples from different ends of the count scale have very different diameters, and on the average samples of adjacent counts, e.g. 48s, will be coarser than those of 50s. However between such adjacent counts there is considerable overlapping of diameters due both to different assessments of the counts and secondly to the considerable variation in mean diameter of wools which are visually well matched for count. It is because of this overlapping of the mean diameters of wools from different count groups that disputes frequently arise. The fact that the mean values for each count group are different is often overlooked. A measure of this overlapping between counts is required to assess the true relationship and value of count and diameter measurements.

In the past, arguments about the relationship of count and diameter have been to some extent academic, as measurement of diameter by the projection microscope was so slow and tedious as to make it unsuitable for general use in commercial transactions. Recently, however, the development of rapid methods of measuring fibre diameter (reviewed by Robinson, 1957) has emphasised again the frequent disparities found in relating count and diameter assessments of a wool.

Most diameter/count relationships have been made on tops on which it is easier to measure fibre diameter than greasy wool. Tables relating count to diameter are reviewed by Hindson and Young (1955): wool tops of 48s count vary from about 32 to 34 μ. As fine fibre is removed as noils during manufacture (Pohle *et al.* 1953) the wool from which the tops are manufactured would be about a micron finer, i.e. from 31 to 33 μ. In practice at the present time, however, a N.Z. importer of English 48s tops would get tops varying in mean diameter from about 35.7 to 37.7 μ, which is much coarser than most of the quoted figures. Such tops would be made from greasy wools of about 35 to 37 μ.

The mean diameter of the samples of 37.5 μ was higher than expected and tops made from these samples would be even coarser. The better-style wools of Trial B were as coarse as those of Trial A and as variable in fibre diameter. Improving style will therefore apparently have little if any effect on reducing within-sample fibre-diameter variability.

The American Society for Testing Materials has laid down tentative specifications (D 419-55T) for the mean fineness of greasy wool of different grades. Its specifications for the stronger counts have always been much finer than the British equivalent. Thus American specifications for 48s greasy wool are for wool with a mean fibre diameter of 31.0 to 32.6 μ. Only one of the samples tested fell within this range and 9 of them would be classified in America as braid wool coarser than 36s.

Within the variation that exists in classers' estimates both within and between countries, concerning the meaning of the present count nomenclature system, and with no basis for standardisation of these

terms, it would seem only reasonable to introduce a universal grade system which has a definite and useful basis, such as the mean fibre diameter of the wools.

The only change involved would be to replace the present count numbers with grade numbers based on the mean fibre diameter in microns. New Zealand wools would be classified in grades from say 20s to 50s using suitable intervals between grades. For example, what we now class as counts 64s, 60s, and 56s would correspond approximately to grades 22s, 24s, and 28s respectively.

On the results reported in this paper, wool of 48s count would be grade 37s. A New Zealand classer therefore on making up a line of straight 48s count wool, would call it grade 37s. That is, he would estimate by means of the usual visual and tactile methods of count assessment (namely crimp, length, diameter, tip and handle) that the wool would have a mean diameter in the vicinity of 37 μ. An American woolman might have classed the same lot of wool as being of 40s count on his scale but with the knowledge that on the average his 40s count wool has a mean diameter of 37 μ, he would also grade it as 37s on the proposed system.

Much preliminary work on many aspects but particularly count-diameter relationship in each country would need to be carried out before such a system could be introduced, but it would have many advantages such as:

(1) As the micron is an internationally accepted and unchangeable unit of measurement, the grading system would be universal, and form a sound basis for national and international trade.

(2) It would be equally applicable to tops and other natural or artificial fibres.

(3) It does not interfere with nor attempt to displace other important aspects of quality such as crimp, handle, colour, or staple length. It only replaces a count number of varied meaning with a grade number closely related to the principal characteristic of the fibres.

(4) It would bring the grading of greasy wool and tops into line with the recently adopted international system for measuring yarn count.

Under both systems the grade number increases with increasing fibre diameter or yarn thickness, that is they are "direct" systems, whereas the present counts for wool and most count systems for yarns are "indirect". (While it would be possible to use the international "Tex" scale for grading greasy wool, a definite disadvantage would be that a geometric scale related to the square of the diameter rather than a simple arithmetical scale would result. A possible unit would be the millitex or weight in milligrammes of a kilometre of fibre. On this scale 70s count greasy wool with a mean diameter of 20 μ would be about 400 millitex, 30 μ wool 900 millitex, and 40 μ wool 1600 millitex).

(5) Over the last 30 years the diameter of the stronger English tops has increased quite markedly, yet the count numbers have remained

unchanged. The proposed system would keep a check on this drift, and thus avoid changing standards. If the tops get stronger the grade numbers would correspondingly increase.

(6) The American grades for greasy wool and tops for Customs purposes are already based on micron measurement. The reconversion of such measurements back to counts based directly on these measurements is carried out only to interpret the micron measurements to the trade.

It is neither proposed nor imagined that the diameter of each lot of wool should be measured. In the first instance however a considerable amount of measurement to relate count and diameter both within and between countries would need to be carried out.

The use of fibre diameter measurements is increasing and will continue to increase in the textile industry both because of the more exacting demands of the modern textile industry, and the need of a reliable "measuring stick" in home and international wool trading.

The introduction of such a system would undoubtedly meet with considerable opposition from the wool industry, and in the first place there would be some problems to solve. But as the present system is not satisfactory and results in many avoidable disputes, it is time some start was made on the introduction of a better system.

The agreement between the mean number of crimps per inch for both trials and for the 28 samples regraded as 48s of 3.2 crimps per inch again emphasises the importance of crimp in assessing count. The method which was used is simple to operate and allows for the variation in crimp frequency along the staple. This variation, which is normal in Crossbred wool, makes it difficult to measure on a crimp wheel. While there was a significant negative correlation between crimp and fibre diameter for this group of wools, the standard error of estimate for predicting diameter from the number of crimps per inch of 2.29 μ is such as to emphasise again the considerable variation in fibre diameter which must be expected in wools which are nominally graded as being of a single count. The standard error is of a similar order for the 28 wools which were regraded as 48s.

It is not proposed to enter here into a detailed discussion of the wide range of breaking loads (14 to 228 lb/g) found for these samples. The results show that there are wide differences in the severity of the lambing break, and these differences would be reflected in the manufacturing performance of the wools and in the strength of the yarns produced from them. Other work on the breaking loads of wools from different sources is in progress, and the results will be published later.

Acknowledgements

The author is indebted to those wool classers and members of the Wool Commission who selected and sent in samples, and to the classers who regraded them.

References

Henderson, A. E.; McMahon, P. R. 1947: *N.Z. J. Sci. Tech. A29:* 22-43.

Hindson, W. R.; Young, F. S. 1955: *Int. Wool Text. Res. Conf., Sydney, 1955.*

Pohle, E. M.; Johnston, D. D.; Ray, H. D.; Manning, W. J.; Mueller, W. A. 1953: *Text. Res. J. 23:* 663-7.

Robinson, A. T. C. 1957: *Texture 4:* 61-7.

Ross, D. A. 1958: *N.Z. J. Sci. 1:* 127-42.

EFFECT ON HONEY BEES OF THIODAN APPLIED TO BROAD BEANS IN A CAGE

By T. Palmer-Jones, Animal Research Station, Department of Agriculture, Wallaceville

(*Received for publication, 17 November 1958*)

Summary

In a cage test, thiodan was sprayed on broad beans at the rate of 56 gallons of a 1% solution per acre.

The application was made an hour before bees could visit the beans, the flowers of which were of maximum attractiveness to bees.

The spray repelled bees for three hours and no bee mortality occurred, although bees collected nectar extensively from the bean flowers when repellency ceased.

The experiment shows that thiodan can be applied to broad beans in flower without causing bee mortality provided it is put down at a time when bees are not flying. It may be safe to apply similarly to flowering white clover pasture but should be used with extreme caution until proved so. It cannot be recommended for application to brassicas in flower until it has been shown safe by a field experiment.

Introduction

New Zealand agriculture is becoming increasingly dependent upon the use of agricultural chemicals. Unfortunately, however, many of these compounds are highly toxic to the honey bees upon which it depends for pollination. The problem therefore has been to find how agricultural chemicals can be used without harming honey bees. Since 1950, the Department of Agriculture has carried out a series of laboratory and field experiments with this aim in view.

Laboratory tests for measuring the toxicity of agricultural chemicals to honey bees were developed by Palmer-Jones (1958) so that these compounds could be quickly and conveniently screened and the most promising ones chosen for field trials. The methods used in field trials have been described by Palmer-Jones and Forster (1958). Field trials will always be necessary before the final decision can be made as to whether or not a new agricultural chemical is safe to bees. But large-scale field trials are costly and time-consuming as they entail spraying at least 8 acres of a crop, hiring an apiary, perhaps paying compensation for bee mortality, and working away from headquarters.

Palmer-Jones (1959) described the construction and use in bee-keeping research of portable cages of nylon insect screening. One type of cage measures 30 × 10 ft and is $6\frac{1}{4}$ ft high, being sufficiently large for bees from a nucleus maintained inside it to collect nectar and pollen normally from an enclosed crop. This crop can be treated with agricultural chemicals and the effect upon the bees studied, so carrying out a field experiment in miniature. The use of such cages, conveniently located at Wallaceville, will considerably reduce the work

involved in testing the effect upon bees of field applications of agricultural chemicals.

On the basis of laboratory tests, Palmer-Jones (1958) considered that the ascending order of toxicity to bees of field applications of a range of agricultural chemicals was toxaphene, strobane, thiodan, Primin, and diazinon. Applications of toxaphene and strobane would be expected to cause little mortality if made after bee activity had ceased; subsequently these predictions were found correct in field experiments (Palmer-Jones *et al.* 1958).

The laboratory tests indicated that thiodan was unsafe to use on crops attractive to bees. But it is much less toxic to bees as a stomach poison than Primin and diazinon, and because of its strong smell might exert repellency under field conditions which would render it safe to use. Thiodan is a sulphur-containing heterocyclic hydrocarbon compound (1,2,3,4,7,7-hexachlor-bicyclo-(2,2,1) - hepten-5, 6 - bisoxymethylene sulphite). Its mammalian toxicity appears to be no greater than that of DDT. It is claimed to have proved consistently good for the control of aphids and other insects (Niagara Chemical Division 1957). If thiodan did not cause bee mortality it might be of value for controlling the clover case-bearer month (*Coleophora spissicornis* and *C. alcyonipennella*?). Accordingly the following cage experiment was carried out with the aim of finding what effect thiodan would have upon honey bees when applied at maximum rates to a flowering crop.

Experimental Procedure

General

Six rows of recently planted broad beans (*Vicia faba*) were enclosed in one of the large cages already described. Growth in the cage was normal and the beans flowered profusely. Shortly before they commenced to flower, a four-frame nucleus of young bees and brood just about to hatch was put in the cage. The bees soon accustomed themselves to conditions in the cage and when the beans flowered they collected nectar and pollen normally, saturating the flowers of the crop. Freshly collected bean honey was observed in the combs of the nucleus.

Before the thiodan was applied, the bees were provided with a supply of water inside the nucleus in place of one outside. An anemometer and thermo-hygrograph were installed in the cage.

Application of Thiodan

Thiodan emulsion (20% W/V) was mixed with water to give a concentration of 1%. This dilution was applied to the beans in the cage from a pressure-operated hand sprayer. Care was taken to ensure that the beans were thoroughly sprayed with thiodan; plants which grew close together were held apart so that the spray could reach the flowers.

The volume of 1% thiodan spray applied to the beans filling the 30 × 10 ft cage was 1750 c.c. This is equivalent to an application of 56 gallons of spray containing 5.6 lb thiodan (1% W/V) per acre of beans.

The spray was applied at 8.50 a.m. on 30 September when conditions were ideal and a dead calm prevailed. There was no dew on the leaves at the time of spraying. Although bees were not leaving the nucleus at the time of spraying, it was closed and opened an hour later. Thus no bees were exposed to the direct action of the spray. The spray had completely dried on the beans by the time the hive was opened.

Counts of Field Bees

The total number of bees on the six rows of beans in the cage was obtained by counting the bees on two rows and multiplying the total by three. The cage covered 1/145 of an acre so the number of bees on the crop in the cage multiplied by 145 gave the number of bees per acre (Fig. 1). The beans in the cage were of even growth.

RESULTS

Counts of Field Bees

Bee counts commenced on 29 September and continued until 3 October, the thiodan spray being applied by 8.50 a.m. on 30 September (Fig. 1). Weather conditions were excellent throughout this period, being suitable for maximum bee activity. Peak bee counts of up to 28,000 bees per acre (192 bees on beans in cage) were recorded, the spray having been applied at a time when the flowers of the crop were of maximum attractiveness to bees. Bees were observed to collect pollen from the flowers of the crop.

At 10.30 a.m., after spraying, although the bees in the cage were very active and some alighted on the leaves of the beans only three were observed to collect nectar from the flowers. The bee count by noon reached 30 bees on the beans (4,350 per acre) but this figure was still well below that usually recorded. It was only in the afternoon that the bee count reached a normal figure (Fig. 1).

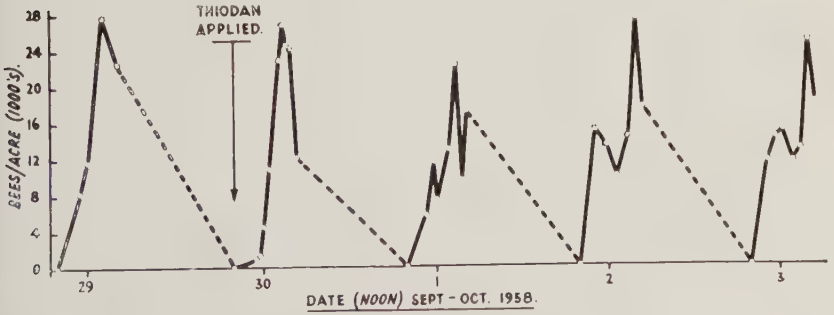


Fig. 1.—Bees per acre of beans.

It is apparent that the thiodan exerted repellency for three hours after spraying.

Effect of Thiodan upon Field Bees

The toxic action of the thiodan was studied by picking bees off the beans and placing them in cages provided with feeders holding sugar syrup; the cages were kept under observation in a 30°c incubator.

On the day the thiodan was applied 20 bees were collected from the flowers at 1 p.m., 25 at 2.30 p.m., and 25 at 4 p.m. The following day 25 bees were collected at noon and 25 at 2.30 p.m. Out of a total of 120 bees collected only one died in 48 hours showing the crop was not toxic to the bees.

Behaviour of Bees on Crop

Although the crop smelt strongly of thiodan on the day of application and the following one, and exhibited repellency for some hours after application, the bees visiting its flowers behaved normally at all times.

Effect of Thiodan on Nucleus

The nucleus was examined on the day of spraying and later. No adverse effect on adult or brood was observed.

Weather

No rain fell during the course of the experiment (29 September to 3 October). Most days were calm, the maximum wind speed recorded being 3.5 m.p.h. Temperature ranged from 36 to 72°F and relative humidity from 40% to 100%. All the days were sunny.

Discussion

Applied at a time when the bean flowers were of maximum attractiveness to bees, the thiodan caused no bee mortality, although laboratory tests had indicated that heavy mortality could be expected. However, laboratory tests do not measure the repellency which an agricultural chemical may exert when applied in the field and thiodan repelled bees for three hours after application.

If used for control of aphids on brassicas, thiodan would not be applied at a rate in excess of 2 lb thiodan in 10 gallons of water per acre. In this experiment it was applied at the much higher rate of 5.6 lb in 56 gallons of water per acre without causing bee mortality.

However, when applied to brassicas, agricultural chemicals are particularly liable to cause heavy bee mortality for the following reasons:

Brassicas attract bees in large numbers in the spring as other sources of nectar and pollen are scarce.

Brassicas are sown very close together and the plants carry many flowers. So while peak bee densities on average white clover pasture reach approximately 4,000 bees per acre, they may exceed 32,000 bees per acre for brassicas.

The brassica flower is a very open one in which sprays and dusts could fall and so come in contact with visiting bees.

Consequently, although thiodan can be applied in safety to broad beans and also perhaps to white-clover pasture in flower, it cannot be recommended for use on brassicas until it has been shown safe by field experiment.

CONCLUSIONS

The experiment shows that thiodan can be applied to broad beans in flower without causing bee mortality, provided it is put down at a time when bees are not flying. It may be safe to apply similarly on flowering white-clover pasture but should be used with extreme caution until proved so. It cannot be recommended for application to brassicas in flower until it has been shown safe by field experiment.

ACKNOWLEDGEMENT

Mr. R. G. Nairn, Animal Research Station, Wallaceville, grew the beans and maintained the bees in the cage.

REFERENCES

Niagara Chemical Division, Food Machinery and Chemical Corporation, Middleport, New York, 1957: Technical Information Niagara Thiodan.

Palmer-Jones, T. 1958: Laboratory Methods for Measuring the Toxicity of Pesticides to Honey Bees. *N.Z. J. agric. Res. 1:* 290-300.

———1959: Construction and Use of Cages in Beekeeping Research. *Ibid. 2:* 242-8.

Palmer-Jones, T.; Forster, I. W. 1958: Agricultural Chemicals and the Beekeeping Industry. *N.Z. J. Agric. 97 (4):* 298.

Palmer-Jones, T.; Forster, I. W.; Line, L. J. S. 1958: Effect on Honey Bees of Toxaphene and Strobane Applied to White Clover Pasture. *N.Z. J. agric. Res. 1:* 694-706.

THE EFFECT OF GRANULE SIZE ON THE EFFICIENCY OF GRANULATED SUPERPHOSPHATE USED AS PASTURE TOPDRESSING

By F. B. Muller, N.Z. Fertiliser Manufacturers' Research Association, Auckland

(Received for publication, 8 December 1958)

Summary

The results of four years' work on the effect of granule size on the efficiency of granulated superphosphate used as pasture topdressing as reflected by yields is reported.

Three sizes of granular superphosphate: 1/32 in. to 1/16 in., 1/16 in. to 1/8 in., and 1/8 in. to 3/16 in. were compared with ordinary commercial superphosphate and commercial granulated double superphosphate, all materials being applied at equal P_2O_5 basis.

An average of 29% phosphate response was observed over the untreated plots over the four-year period. During that time the medium-sized granules gave a four per cent increase in yield over ordinary superphosphate, and a six per cent increase over the coarse granules. Statistically, these figures are not significant, but they indicate a trend toward better utilisation of the P_2O_5 from medium granules. During the fourth year the fine granules gave significantly higher yields than the coarse.

A granulated superphosphate with granules ranging from 1/32 in. to 3/16 in. and with over 50% between 1/16 in. and 1/8 in. would be at least as effective agronomically as ordinary superphosphate for use as topdressing on pastures.

Introduction

Overseas literature on the use of granulated superphosphate has been concerned mainly with the use of this material on arable crops, where results are often significantly in favour of the granulated materials, especially when drilled in with cereal crops. References on the use of granulated superphosphate as a topdressing are not so numerous but in general consider the material to be at least as good as the powdered form.

In pot trials when used as a topdressing for crimson clover Fruhstorfer (1955) obtained as good results with granular as with powdered superphosphate at two levels of P_2O_5. He further recommended that the granule size be between 1 mm and 5 mm (approximately 1/32 in. to 3/16 in.) with 5% variation in over-size and fines. The results of an experiment by the German Fertiliser Manufacturers' Association on granule size is reported (Sauchelli 1950) but there again the materials were applied to arable crops either broadcast or drilled in. The highest yields were from the 2 to 5 mm granules. Valente Almeida *et al.* (1957) in Portugal substantiate this statement in favour of 2 to 5 mm granules (1/16 in. to 3/16 in.).

In a field experiment on grassland in France, Garola (1951) showed that granulated superphosphate had a very slight though not

significant advantage over powdered superphosphate when used as a topdressing. The size of the granules was not given.

In New Zealand, the first published accounts on the use of granulated superphosphate were by Miller (1951) and Greenall (1952) who described an experiment conducted in 1949 using granulated superphosphate applied from the air when commercial powdered superphosphate was not successful. The granulated material was spread from a converted military aircraft and the ground distribution pattern measured. The following year a mowing trial comparing different-sized granules was started both at the Rukuhia Soil Research Station and the Marton Experimental area. The results were reported by Lynch and Jessop (1953). The granules were screened from ordinary commercial superphosphate into coarse granules retained on a $\frac{3}{8}$ in. screen and passing $\frac{1}{2}$ in., fine granules retained on $\frac{1}{4}$ in. and passing $\frac{3}{8}$ in., and "fines" passing $\frac{1}{8}$ in. These were compared with ordinary superphosphate passing $\frac{1}{4}$ in. screen. In both trials pasture production records over the trial period showed no significant difference between the various superphosphate treatments, though the finer materials gave generally quicker responses.

The trial described in this paper was conducted to determine the agronomic value of experimental lots of granulated superphosphate made in New Zealand.

EXPERIMENTAL

The trial was initiated during the winter of 1953 at the N.Z. Fertiliser Manufacturers' Research Association plots. The soil where the trial was laid out is classified as Papatoetoe silt loam, a water-sorted volcanic ash (N.Z. Soil Bureau 1954). It is a well drained granular loam of pH 5.8, perfectly level where the trial was conducted. The annual rainfall averages about 48 inches.

The area had been in grass for some thirty years before the trial was begun and had not been topdressed for at least ten years. From 1950 to 1953 the area had been maintained as a lawn, the clippings being removed with each cut. The area was selected for this trial because of the low phosphate supply. In August 1953 the plots were laid out, each plot being 38 ft × 4 ft. There were four replicates for each treatment.

On a per-acre basis each plot received three cwt of superphosphate (21% water soluble and 24% total P_2O_5), or granulated double superphosphate (44.3% and 49.5%) on an equal P_2O_5 basis in August 1953. In addition each plot received one cwt muriate of potash and two cwt dried blood. In April 1955 and again in October 1956 the plots were topdressed with phosphates and potash but no nitrogen. During the four years' trial the plots received the equivalent of nine cwt of superphosphate or 242 lb of P_2O_5.

The materials were applied at intervals of one and a half years instead of yearly in order to study the residual effects of the various-sized granules in comparison with ordinary superphosphate.

The following treatments were made:

Treatment	Particle range	
	Passes	Retained on
1. Control—no phosphates	—	—
2. Granulated superphosphate—coarse granules	3/16 in.	1/8 in.
3. Granulated superphosphate—medium granules	1/8 in.	1/16 in.
4. Granulated superphosphate—fine granules	1/16 in.	1/32 in.
5. Ordinary superphosphate	—	—
6. Granulated double superphosphate	3/16 in.	1/32 in.

The ordinary superphosphate was a commercial sample with nearly 80% passing through a 1/32 in. screen, while the granulated double superphosphate had nearly 75% of the granules in the 1/16 to 1/8 in. range. The screen analysis of these two materials follows:

	Ordinary Commercial Super-phosphate Per Cent	Granulated Double Super-phosphate Per Cent
Passing 1/4 in. screen, retained on 3/16 in.	0.0	0.1
" 3/16 in. " " " 1/8 in.	0.5	16.8
" 1/8 in. " " " 1/16 in.	5.8	74.2
" 1/16 in. " " " 1/32 in.	14.6	8.1
" 1/32 in. "	78.8	0.5

The commercial superphosphate was a locally made product, the granulated superphosphates were made locally especially for this trial, and the granulated double superphosphate was of Dutch manufacture.

The sward consisted mainly of ryegrass, white clover, and paspalum. The plots were harvested by mowing, the clippings being discarded to deplete further the soil phosphate supply. After weighing the clippings from the individual plots, samples were obtained for moisture determination.

RESULTS

The results of this experiment are summarised in Table 1. Fig. 1 gives the percentage yield for the three granule sizes compared with ordinary superphosphate for the 48 individual harvests.

While it is only during the final year of the experiment that significant differences were obtained, there are several trends throughout the harvest data which indicate that granules ranging between 1/16 in. and 1/8 in. give consistently higher yields over long periods.

When topdressed for the first time, there was an immediate phosphate response which increased during the year. Ordinary super-

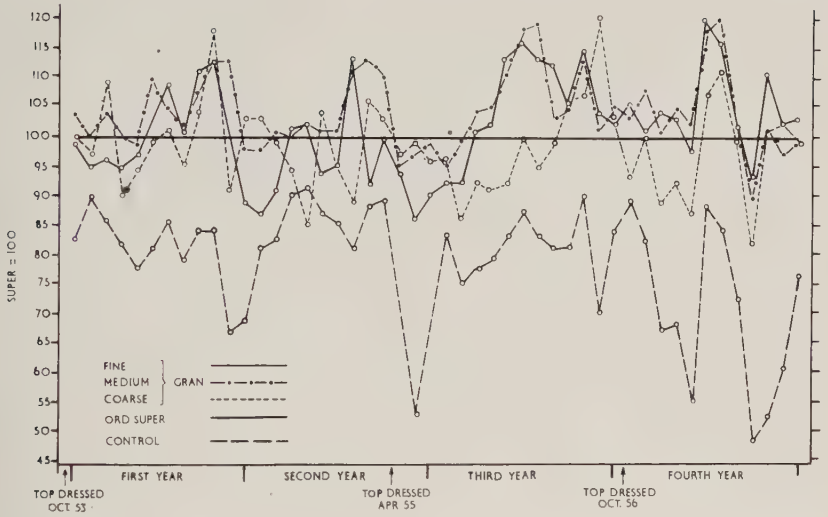


Fig. 1.—Relative yields of granulated supers compared with ordinary super = 100.

TABLE 1. SUMMARY OF DRY MATTER YIELDS (lb/ac) RELATIVE TO SUPERPHOSPHATE (100)
(Total of 12 harvests per year)

	Oct 53–Sep 54		Sep 54–Oct 55		Oct 55–Oct 56		Nov 56–Oct 57		Total Four Years	
	Yield	Rel	Yield	Rel	Yield	Rel	Yield	Rel	Yield	Rel
1. Control—No Phos.	3942	81	4980	79	6585	80	5397	71	20,904	77
2. Gran. Super Coarse	4857	100	6176	98	7986	97	7316	96	26,335	98
3. Gran. Super Medium	5060	104	6410	102	8621	105	7837	103	27,928	104
4. Gran. Super Fine	4869	100	6036	96	8501	104	7941	104	27,347	101
5. Ordinary Super	4853	100	6295	100	8203	100	7603	100	26,954	100
6. Double Super (Gran.)	4957	102	6009	95	8231	100	7523	99	26,720	99
L.S.D. at 5% level	355	—	808	—	824	—	566	—	2,145	—

phosphate fell off in effectiveness in comparison with the granulated superphosphates as the year progressed.

By the beginning of the second year, the phosphates applied had been practically used up or fixed, and in some cases the control plots gave as good yields as some of the phosphate-treated plots. As a whole,

the medium-sized granules gave consistently higher yields, while the other granulated superphosphates were somewhat erratic.

When topdressed for the second time in April 1955, ordinary superphosphate gave an immediate response which lasted well into the winter, but by the summer of 1955-56 ordinary superphosphate was well below the yields from all the treatments except from granulated superphosphate coarse granules. By May 1956, the coarse granular material had picked up and was also better than ordinary superphosphate.

Following the final topdressing in October 1956, there was a strong response from the two finer granulated superphosphates and ordinary superphosphate until mid summer of 1957, when all treatments were again better than ordinary superphosphate. In the autumn, ordinary superphosphate had made some recovery and by the end of the trial all treatments gave about the same yield over control. During the final year of the trial the fine granules were significantly better than the coarse. Statistically there were no significant differences between the remainder of the treatments although the medium-sized granules also gave good results.

In general over the four-year period the effectiveness of the coarse granules fell off compared with ordinary superphosphate while the fine granules increased in effectiveness.

During the first three years there was a consistent increase in phosphate response on an average of the treated plots and by the fourth year the phosphate response had jumped to over 40%, indicating that the phosphate deficiency on the control plots was being aggravated by the continued removal of all herbage.

Soil samples were taken at the end of the trial for pH determination. The average of the treated plots was 5.85 and of the control plot 5.86. These figures are not materially different from the figure of 5.82 obtained at the beginning of the trial.

Discussion

The increase in phosphate response from 25% to 40% over control from the first year to the fourth year of the trial indicates that higher yields might have been obtained by increasing the amount of P_2O_5 added, or by more frequent application of the phosphates. Also the fact that phosphate responses begin to drop off within a year of each topdressing shows that 81 lb of P_2O_5 per year was barely enough to keep ahead of phosphate demand, and over a period of $1\frac{1}{2}$ years was not sufficient.

Under these conditions any real differences in the availability to pasture of the different materials should have been brought out. The fact that over the four-year period the difference in yields is so small shows that there is no loss in effectiveness of the superphosphate by granulating. Rather there appears to be a trend indicating that granules from $\frac{1}{32}$ in. to $\frac{1}{8}$ in. may have a stabilising effect; that is, may release P_2O_5 rather more slowly at first, but maintain a source of available

phosphates for a longer period. Ordinary superphosphate on the other hand tends to fluctuate more widely in relation to the control plots. The "fines" in ordinary superphosphate do not necessarily bring out a quicker response than granular materials, as is shown by the yield figures following the October 1956 topdressing.

Rainfall appears to have considerable influence on the relative yields of pastures treated with these materials. While 48 inches per year is the average for the district, wide variations from that norm can be expected. Not only the total precipitation but the distribution can have an influence on the availability of these materials. The total amount of rainfall and its distribution is reflected in the yields during the four years of the trial. The rainfall for the trial period was:

October	1953–September	1954	inc.	44.06 in.
,,	1954 ,,	1955	,,	42.83 ,,
,,	1955 ,,	1956	,,	64.75 ,,
,,	1956 ,,	1957	,,	43.13 ,,

The monthly distribution of rainfall for the periods following topdressing are given in Table 2.

TABLE 2. MONTHLY RAINFALL FOLLOWING TOPDRESSING

Topdressing of:	21 August 1953	26 April 1955	17 October 1956
Rainfall during	Aug 3.85 in.	April 1.12 in.	Oct 2.69 in.
	Sep 1.55 in.	May 3.96 in.	Nov 4.15 in.
	Oct 2.87 in.	June 6.59 in.	Dec 4.78 in.
	Nov 2.13 in.	July 5.05 in.	Jan 0.17 in.
	Dec 3.38 in.	Aug 4.08 in.	Feb 2.51 in.
	13.78 in.	20.80 in.	14.30 in.

The amount of rain falling in the four months prior to topdressing in April 1955 was 9.21 in.; not very high considering that this amount fell during the summer months when the ground is usually dry and evaporation is at its peak. The rainfall immediately following application was not heavy. This could account for the higher yields with ordinary superphosphate following this topdressing.

The amount of rainfall following the other two topdressings, which, in addition, occurred at the end of the winter period, was sufficient to bring the yields of the granulated materials up to the level of ordinary superphosphate. The rainfall for the four-months' period May-August 1953 was 27.61 in. and July-October 1956 was 23.70 in., more than enough rain over the short-day periods to leave the soil saturated.

The coarse granules as a whole have given poorer results, especially during the last year of the trial, and the decrease in effectiveness over the trial period has been nearly constant. By the fourth year the coarse granules gave significantly lower yields than the fine granules. This

would indicate that granules larger than the coarse material used in this trial will not give satisfactory results when used as a topdressing at the rate of P_2O_5 used.

Foreign workers have found that up to 5 mm (3/16 in.) there was no decrease in yield, while the New Zealand work previously described might indicate that from a long-term view all granule sizes up to 1/2 in. size give equivalent results. Our results show that with granules larger than 1/8 in. there is liable to be a decrease in yield in comparison with the finer sizes. When used at a reduced rate of P_2O_5 Fruhstorfer (1955) did find a slight decrease in yield using 2 to 5 mm material as a topdressing on crimson clover and maize. It is only on doubling these rates that the coarser material exceeded the powder. Evidently at the lower rates of P_2O_5 the coarser granules are not able to furnish optimum amounts of phosphates, probably due in part to the distance between granules as the larger sizes are approached. If larger-sized granules such as over 3/16 in. diameter are used, higher rates of P_2O_5 will have to be used to compensate for the decrease in number of particles to cover a certain area.

Conclusions

1. Granulated superphosphate with granule size between 1/32 in. and 1/8 in. has given as good if not better yields than ordinary superphosphate when topdressed on pasture.
2. Powdered superphosphate will give quicker response if the topdressing is done under dry conditions. Under wet conditions the granular product has given quicker response than ordinary superphosphate as well as higher yields in general.
3. There tends to be a falling off in yields if the granules are all larger than 1/8 in. in diameter when marginal amounts of phosphates are used.
4. A granulated product between 1/32 in. and 3/16 in. where over half of the material lies between 1/16 in. and 1/8 in. would give results at least equal to ordinary superphosphate when used under moist conditions.

Acknowledgements

The author wishes to thank the Management Committee of the N.Z. Fertiliser Manufacturers' Research Association for permission to publish this paper, Dr. B. W. Doak for his assistance and helpful suggestions, and the staff of the Association for their help in running this trial.

References

Fruhstorfer, A. 1955: Influence of Granule Size and Product on the Action of Phosphates. *Agric. Chem. 10 (5):* 30-32.

Garola, J. 1951: Comparison of the Effect of Phosphoric Acid in the Form of Powdered and Granular Superphosphate. *Bull. Docum. Ass. int. Fabr. Superph. 9:* 10-13.

Greenall, A. F. 1952: Aerial Topdressing Trials: Equipment and Flying Techniques Used and Measurement of Distribution of Superphosphate. *N.Z. J. Sci. Tech. A33 (6):* 54-68.

Lynch, P. B.; Jessop, H. N. 1953: Granulated Superphosphate—Recent Research Work. *N.Z. J. Agric. 86:* 55.

Miller, J. 1951: Measurement of the Distribution of Granular Superphosphate from Aeroplanes. *N.Z. J. Sci. Tech. A33 (2):* 17-27.

N.Z. Soil Bureau 1954: General Survey of the Soils of North Island, New Zealand. *N.Z. Soil Bur. Bull. n.s. 5.*

Sauchelli, V. 1950: Granule Size vs Crop Response. *Amer. Fert. 113 (6):* 8-9.

Valente Almeida, L. A.; Balbino, L. R.; Nunes, M. L. 1957: The Effect of Granular Superphosphate on a Granitic Soil. *Bull. Docum. Ass. int. Fabr. Superph 22:* 3-10.

CONSTRUCTION AND USE OF CAGES IN BEEKEEPING RESEARCH

By T. Palmer-Jones, Animal Research Station, Department of Agriculture, Wallaceville

(Received for publication, 16 October 1958)

Summary

Pollination research in New Zealand is reviewed.

The construction of portable field cages is described. These are made of panels of nylon insect screening supported on a "Dexion" framework.

One type of cage measures 30 × 10 ft and is 6¼ ft high, being sufficiently large for bees from an enclosed nucleus to visit flowers inside normally and collect nectar and pollen. It is used in pollination research for either saturating an area of a crop or pasture with bees or excluding them altogether; the effect upon bees of agricultural chemicals sprayed and dusted over enclosed crops can also be studied.

The other type of cage is much smaller, measuring 5 × 2½ ft, with a height of 2½ ft. It is used to exclude bees from pastures and crops in pollination studies.

These cages are easily transported and assembled, and, because of their sturdy construction, have proved satisfactory for use under our windy climatic conditions.

Introduction

The New Zealand seed-growing industry is an important one, the main seeds produced being white clover, the brassicas, lucerne, and red clover.

Hadfield and Calder (1936) investigated the pollination of lucerne by hive and bumble bees and Palmer-Jones and Forster (1958a) published observations on the pollination of a lucerne crop.

Montgomery (1952) and Gurr (1955) studied the pollination of red clover by bumble bees and Cumber (1953) reviewed the various factors that would govern the use of bumble bees for the pollination of red clover in New Zealand. Forster and Hadfield (1958) studied the effectiveness of honey bees compared with bumble bees as pollinators of Montgomery red clover.

It is apparent from this review that most pollination research in New Zealand has been concerned with bumble bees. Although bumble bees are valuable pollinators in the hill country areas, they are of little account in the plains and coastal regions where most of our seed crops are grown (Palmer-Jones and Forster, 1958a; Palmer-Jones 1959). Consequently, as pointed out by Palmer-Jones (1959), although the white-clover seed industry is virtually entirely dependent upon honey bees for pollination, no experimental work has yet been undertaken to find the most effective way in which to use them as pollinators. Work has certainly been carried out on lucerne and red clover but, because

satisfactory pollination of these plants is difficult to achieve, a great deal still remains to be done before we will be in a position to make definite recommendations regarding their pollination requirements. In the case of fruit trees, apart from work on the pollination of plums undertaken by Roberts and Congdon (1955), the field is virtually untouched.

A comprehensive research programme, which will extend over several years, has been planned by the Department of Agriculture with the aim of finding how to increase the yields of seed crops by the most effective use of honey bees. The pollination of fruit trees and clover pasture, and the part played by bumble bees as pollinators in hill country areas, will also be investigated.

In such work it is essential to exclude bees from control areas of a crop or pasture so that their value as pollinators can be assessed. It is also frequently of help to be able to saturate an area with bees. These and other aims can be attained only by the use of cages; thus cages can be used for experiments in the Mackenzie Country which has bumble bees but as yet practically no honey bees. The pollination of clovers inside cages there could be compared with that outside, so measuring the value of the bumble bees as pollinators. The value, as pollinators, of honey bees plus bumble bees, or honey bees alone, could also be assessed.

Many large-scale field experiments designed to test the effect of agricultural chemicals upon honey bees have been carried out by the Department of Agriculture (Palmer-Jones and Forster 1958b). In such trials it is necessary to spray at least eight acres of a crop or pasture, hire an apiary, and undertake prolonged work away from one's headquarters. It is possible to reduce such work considerably by the use of cages; enclosed crops are sprayed with the agricultural chemicals under test and the effect of these upon bees visiting the crops from nuclei maintained inside the cages studied.

Hadfield and Calder (1936) used cages measuring 12 × 6 ft, with a height of 6 ft, in experiments on the pollination of lucerne. The cages were not portable and their wooden framework was covered with mosquito netting. It was observed that honey bees from nuclei inside the cages were not contented and spent most of their time attempting to escape. This behaviour was probably due to the smallness of the cages.

Field cages described by Pedersen *et al.* (1950) have been used in the U.S.A. These are made of nylon screening supported on a framework of half-inch electrical conduit. Although such cages have proved very satisfactory in America, they would be unsuitable in New Zealand where our frequent high winds make it essential to use a much sturdier type of construction.

Two types of field cage have been developed for use under our conditions. The first is sufficiently large for bees from an enclosed nucleus to visit flowers inside normally and collect nectar and pollen. The second is a much smaller cage, which is used in larger numbers to exclude bees from pastures and crops in pollination studies.

LARGE CAGES

Materials

Framework. This is constructed of "Dexion" slotted angle aluminium alloy supplied in 10-ft lengths, which are joined together by bolting them on to sleeves of the same material. Dexion is light, weather resistant, and easily bolted together.

Six pieces of "Dexion" are bolted at an angle between roof and vertical members of the framework; a double thickness is bolted down the mid line of the roof and a length is bolted midway across the bottom of the cage. These members maintain the rigidity of the cage (Figs. 1, 2).

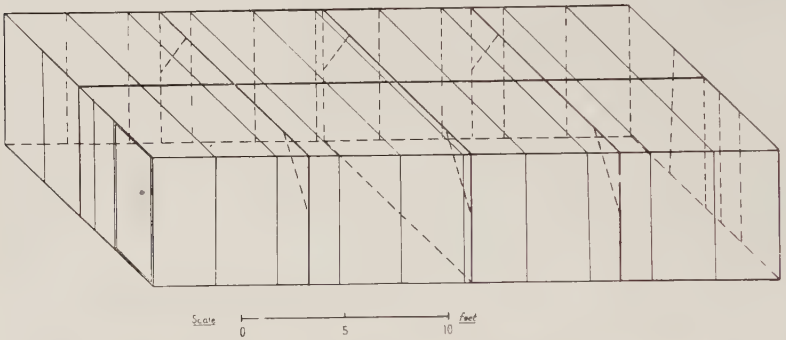


Fig. 1.—Arrangement of framework and frames in large cage.

Fig. 2.—Framework of large cage.

Screening. The framework is covered with frames of "Tygan" nylon insect screening which is supplied in 100 × 3 ft rolls. Clear nylon screening is used as it interferes least with the normal passage of light. The mesh is 12 per inch as this offers little hindrance to the passage of air; temperature and humidity then only build up slightly inside the cage so that plant growth is normal (Fig. 3).

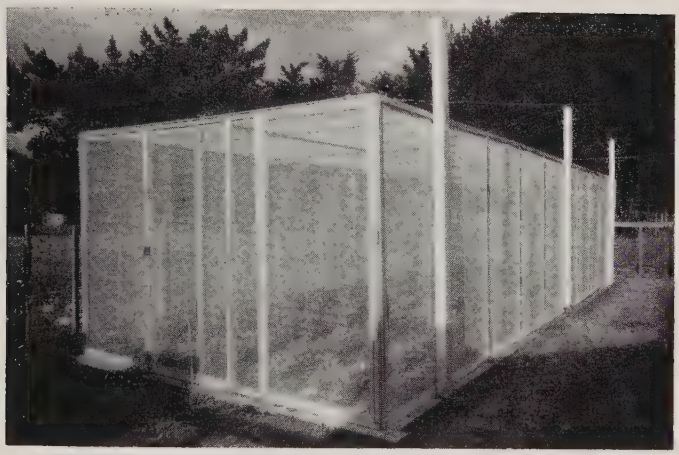


Fig. 3.—Large cage completely assembled.

Frames. The screening is held in frames which are bolted to the framework. The frames are made of extruded aluminium frame section which is cut to size, mitred, and put together with inner corners. The edge of the nylon mesh fits into a groove which runs round the inside of the frames. It is kept firmly in place in the groove by P.V.C. tubing forced in on top of it. The frames are bolted to the framework with $1\frac{1}{4}$ inch hexagonal-head engine bolts, two washers being used per bolt. Metal clips are used to prevent adjacent frame edges springing apart and leaving gaps.

Dimensions

The cages measure 30 × 10 ft and are $6\frac{1}{4}$ ft high.

The roofs are covered by twenty 3 ft × 4 ft $11\frac{1}{4}$ in. panels. Twenty-six 3 × $6\frac{1}{4}$ ft panels plus two $6\frac{1}{4}$ ft × $11\frac{1}{2}$ in. cover the sides. These measurements are all exterior. Fig. 1 shows the arrangement of the panels.

A panel with hinges and a catch is let into one of the end panels to serve as a door.

Transport and Assembly

Each dissembled cage is packed for transport in strong wooden cases with screw-down lids.

The bottom members of a cage are laid on creosoted boards levelled with a spirit level which is also used during assembly to check that the uprights are vertical.

If these boards are nailed to pegs driven in flush with the surface of the ground, the bottom members of the cage can be nailed to them without fear of any movement.

Twelve eye bolts are fixed round the top of a cage so that it can be anchored down. This is done by running flexible galvanised washing

line from the eyebolts to 30-in. lengths of one-inch galvanised piping driven into the ground. The lengths of piping have hooked key clamps attached to their tops so that the wires are easily attached.

Cages are always assembled in as sheltered a place as possible, but if left up for any length of time it is advisable to sink three heavy pieces of timber vertically in the ground so that they rest against the side most exposed to the wind. The framework there can then be bolted or tied to the uprights which give it rigidity and prevent racking. This method has proved perfectly satisfactory and is much cheaper and simpler than adding sufficient internal members to the framework to make it strong enough to dispense with outside support.

Cost and Time of Erection

A cage costs approximately £200. Two men can easily erect it in a day.

Small Cages

These are made of the same materials as the large cages. They measure 5 × 2½ ft, and are 2½ ft high. In the field, wooden stakes are driven in the ground against each side to prevent movement by wind or stock. The cost of a cage is approximately £16.

The cages are sufficiently high for use on red- and white-clover seed crops and pasture. When necessary, the roof frame of one cage can be easily unbolted and another cage bolted on top of it to give increased height so that brassicas and small bushes may be covered; wire stays are then necessary for support (Figs. 4, 5). Side frames can also be removed and two or more cages bolted together to cover larger areas of crop.

Fig. 4.—Small Cage (single unit).

Provision of Bees in Large Cages

Shortly before the enclosed crop commences to flower, a nucleus is placed inside the cage to provide bees. The nucleus is formed by taking

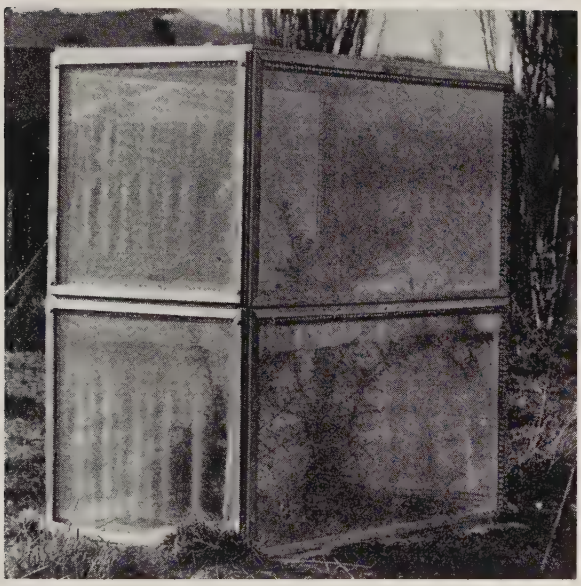


Fig. 5.—Small cage (double unit).

from a hive four frames of brood on the point of hatching, a vigorous laying queen, and adult bees. It is removed some distance from the parent hive so that all the field bees return home, leaving only young bees with the hatching brood. The nucleus is then put in the cage where the bees, none of whom have previously foraged, soon accustom themselves to the conditions and collect nectar and pollen normally, saturating the flowers of the crop.

Although some field bees will fly round the top of the cage and attempt to leave, no undue mortality of bees occurs and the bees in the nucleus behave as if it is unconfined.

The nucleus is supplied with honey and pollen. Water is provided inside the cage.

DISCUSSION

Both types of cage are relatively cheap, portable, easily assembled and taken down, durable, and can be used for many purposes in bee-keeping research. They could also be used as insectaries and in horticultural research.

ACKNOWLEDGEMENTS

The author is grateful for help given in assembling these cages by Messrs. G. L. Jeffery, Instructor in Apiculture, Oamaru, and R. G. Nairn, Technician, Animal Research Station, Wallaceville, both of the Department of Agriculture. Mr. Jeffery drew Fig. 1 and Mr. A. L. Bryant, Photographer, Animal Research Station, Wallaceville, took the photographs used as illustrations.

References

Cumber, R. A. 1953: Some Aspects of the Biology and Ecology of Humble Bees Bearing upon the Yields of Red Clover Seed in New Zealand. *N.Z. J. Sci. Tech. B34:* 227.

Forster, I. W.; Hadfield, W. V. 1958: Effectiveness of Honey Bees and Bumble Bees in the Pollination of Montgomery Red Clover. *N.Z. J. agric. Res. 1:* 607-19.

Gurr, L. 1955: A Note on the Efficiency of *Bombus terrestris* (L) as a Pollinator of Lucerne (*Medicago sativa L.*) *N.Z. J. Sci. Tech. A37:* 300.

Hadfield, J. W.; Calder, R. A. 1936: Lucerne (*Medicago sativa*). Investigations Relative to Pollination and Seed Production in New Zealand. *N.Z. J. Sci. Tech. 17:* 577.

Montgomery, B. E. 1952: Bumble Bees and Red Clover in New Zealand. *N.Z. Sci. Rev. 10:* 47.

Palmer-Jones, T. 1959: Organisation of Pollination Service for Farm Seed Industry. *N.Z. J. Agric 98:* 17-24.

Palmer-Jones, T.; Forster, I. W. 1958a: Effect on Honey Bees of D.D.T. Applied from the Air as a Spray to Lucerne; Notes on Lucerne Pollination. *N.Z. J. agric. Res. 1:* 627-32.

————1958b: Agricultural Chemicals and the Beekeeping Industry. *N.Z. J. Agric. 97:* 298.

Pedersen, Marion, W.; Todd, F. E.; Lieberman, F. V. 1950: A Portable Field Cage. *U.S. Dep. Agric. Bur. Ent. Bull. ET-289.*

Roberts, D.; Congdon, N. B. 1955: The Relationship of Nectar Secretion (Volume) and Sugar Concentration to Insect Pollination of Plums (*Prunus* spp.) *N.Z. J. Sci. Tech. A37:* 196.

DISCOVERY OF THE GENUS *Basidiophora* Roze et Cornu IN NEW ZEALAND

By Hasan Jafar (Pakistan), Ph.D. Student under Colombo Plan, Botany Department, University of Auckland, New Zealand

(*Received for publication, 16 October 1958*)

Summary

The genus *Basidiophora* belonging to the family Peronosporaceae was discovered on *Felicia bergeriana* L. in New Zealand. The species was determined and described.

Introduction

Early workers have recorded the occurrence of certain genera of Peronosporaceae in New Zealand: those recorded to date are *Bremia, Peronospora, Plasmopara,* and *Pseudoperonospora.* Information is still lacking concerning the existence of other important genera, e.g. *Bremiella, Kawakamia, Rhysotheca,* and *Sclerospora* in this country.

In the present paper an account is given of the occurrence of *Basidiophora entospora* Roze et Cornu, a species of a genus not formerly known from New Zealand.

Basidiophora Roze et Cornu (1869)

Mycelium branched and intercellular, forming vesicular haustoria which absorb nutrition from the host cells; conidiophores simple, clavate, stout, with swollen apex bearing short sterigmata: each sterigma bearing a single conidium; conidia papillate and globose, and germinate by formation of biflagellate zoospores; oospores globose and coloured.

Type species:—*Basidiophora entospora* Roze et Cornu

Basidiophora entospora Roze et Cornu (1869)

Syn. *Plasmopara entospora* Schröter (1889)
Gilletia spinuligera Sacc. et Therry, sec. Saccardo (1888)
Peronospora simplex Peck (1881)

Mycelium branched, non-septate, hyaline, coenocytic, intercellular, 6–12 μ wide; haustoria vesiculiform, small; conidiophore (Fig. 1a, b) unbranched, erect, stout, clavate, hyaline, swollen at apex, emerging from stomata singly or in groups (2 to 6), 95–180 μ long, base 3–8 μ wide, apex 10–22 μ wide; sterigmata borne on the swollen apex of conidiophores, short, numerous, 3–7 $\times$ 2·5 μ; conidia (Fig. 1c) borne singly on the sterigmata, globose, tips papillate, hyaline, 22–28 $\times$ 22–26 μ, germination by means of zoospores; zoospores small, round to oval, biflagellate, 4–5 $\times$ 3–4 μ: oospores (Fig. 1d) embedded in host tissue, yellowish, globose, 42–48 μ.

The morphological characters were compared with the descriptions of Massee and Massee (1913), Rabenhorst (1892), Saccardo (1888),

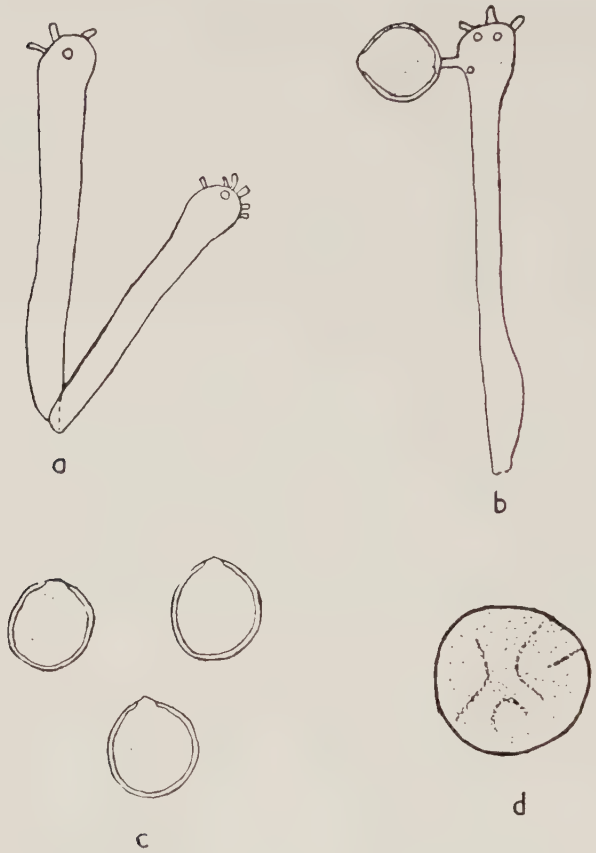


Fig. 1.—*Basidiophora entospora*. a. Conidiophores bearing sterigmata on the swollen apex. b. Conidiophore with a conidium attached to a sterigma. c. Conidia. d. Oospore. All × 500.

and Viennot-Bourgin (1956) and were found to be in agreement with descriptions of the species given by these workers.

General distribution: Belgium, Britain, France, Germany, Italy, North America, and New Zealand. Wilson (1908) has reported it on *Leptilon canadense* (L.) Britton in North America. It has been found on leaves of *Erigeron canadense* L. in the countries where recorded.

Local distribution: Auckland. On leaves of *Felicia bergeriana* L.

Symptom: The first indications of infection on *Felicia bergeriana* L. appear as scattered light-green to yellowish areas on the upper surface of the leaves. Corresponding to these discoloured areas, the fungus develops on the lower surface as patches of downy white. Severely infected leaves turn brown and die. Infected plants remain stunted in growth, the branches droop and the flowers are not of normal size.

ACKNOWLEDGEMENTS

The writer wishes to express his grateful thanks to Dr. V. J. Chapman, Professor of Botany, University of Auckland, for his helpful criticism and to Miss J. Dingley of Plant Diseases Division, Department of Scientific and Industrial Research, New Zealand, for useful suggestions.

The writer is also indebted to Miss R. Gribble for typing the manuscript.

REFERENCES

Massee, G.; Massee, I. 1913: "A Synopsis of the Families Peronosporaceae, Erysiphaceae, Uredinaceae and Ustilaginaceae". 229 pp. Dulau, London.

*Peck, Ch. H. 1881: *Hedwigia 20:* 154.

Rabenhorst, L. 1892: *Kryptogamenflora 6 (4):* 423-5.

*Roze and Cornu, M. 1869: *Ann. Sci. Nat. 5, 11:* 84.

Saccardo, P. A. 1888: *Syll. fung. 7:* 239.

*Schröter, J. 1889: "Kryptogamen-Flora von Schlesien". Vol. 3, Pt. I, p. 237. Breslau.

Viennot-Bourgin, G. 1956: "Mildious, Oidiums, Caries, Charbons, Rouilles Des Plantes De France." Encyclopédie Mycologique, 317 pp. Editions Paul Lechevalier, Paris.

Wilson, G. W. 1908: Studies in North American Peronosporales. *Torrey Bot. Club Bull. 35:* 547.

(*Papers not seen.)

A NOTE ON A COMPARISON BETWEEN THE CARCASS COMPOSITION OF ROMNEY AND CHEVIOT MATURE EWES

By A. H. Kirton, R. A. Barton, and E. Cresswell,*
Massey Agricultural College, Palmerston North

(Received for publication, 1 October 1958)

Summary

Four each of Cheviot and Romney mature ewes of equal group mean live weights were compared by carcass analyses. It was shown that the Cheviot carcasses contained significantly more protein and water. This was related to the heavier, though statistically non-significant weight of dissectible muscular tissue in the Cheviot carcasses.

Introduction

Few studies of the suitability of various breeds of sheep for differing environments have been undertaken. Investigations thus far have been concerned mainly with production characteristics such as lambing percentage, survival of animals, fleece weight, and wool quality, while the physiological characteristics and carcass or body composition of the breeds being compared have been largely neglected.

During the past ten years some work at this College has been directed at obtaining comparative data of the production performance under both hill and lowland conditions of Romney and Cheviot sheep and the various crosses between them. Lately, fundamental physiological data on these two breeds have been collected (Cresswell, unpublished data) and at the end of these experiments animals were available for slaughter and carcass analyses.

Material and Methods

Four mature stall-fed Romney and five stall-fed Cheviot ewes of similar live weight were slaughtered and their carcasses, after weighing, were frozen and held in cold storage for 9 months. In order that each breed group could be compared on a basis of equal live weight, the lightest Cheviot (70 lb live weight) was discarded; thus data analyses were based on four Romney and four Cheviot carcasses.

Each carcass, while still frozen, was divided down the backbone with a bandsaw. The right side, after being totally sliced and the slices minced, was analysed chemically using the methods described by Kirton and Barton (1958) except, however, that in the present experiment individual carcass fat corrections and ashes were determined. The leg, half loin, and 9–10–11 rib cut from the left side of the carcass were each dissected into their physical components (Kirton and Barton 1958). These sample joints have been found to be closely related to total carcass composition (Barton and Kirton 1958; Kirton 1957).

* Present address: Department of Animal Husbandry, Utah State University, Logan, Utah, U.S.A.

RESULTS

Slaughter and chemical-compositon data are presented in Table 1 and dissection data are shown in Table 2. The carcasses were paired (one of each breed) in ascending order of weight and the *t* test was used to test the significance of the differences between the breed means.

TABLE 1. SLAUGHTER AND CHEMICAL COMPOSITION DATA
(4 animals per breed)

	Romney		Cheviot		
	Mean	S.D.	Mean	S.D.	
Live weight (lb)	101.5	10.3	101.0	16.4	
Hot carcass weight (lb)	45.6	4.4	47.2	6.7	
Dressing percentage (a)	45.0	2.0	47.0	2.2	n.s.
Shrinkage (lb) (b)	4.6	1.1	4.3	1.2	
Chemical fat (lb)	10.7	2.7	9.6	4.1	
Water (lb)	26.4	1.6	28.0	1.7*	
Protein (lb)	6.3	0.7	7.4	0.8**	
Ash (lb)	2.2	0.1	2.3	0.2	

* Difference significant at 5% level.
** Difference significant at 1% level.
(a) Hot carcass weight/live weight at slaughter.
(b) Hot carcass weight—frozen carcass weight.

TABLE 2. DISSECTIBLE COMPONENTS OF THE LEG, HALF LOIN, AND 9–10–11 RIB CUT
(4 animals per breed)

	Romney			Cheviot		
	Mean Weight (g)	S.D.	%	Mean Weight (g)	S.D.	%
Leg	2179	250		2303	248	n.s.
Fatty tissue	388	87	17.8	325	123	14.1
Muscular tissue	1434	189	65.8	1593	100	69.2 n.s.
Bone	263	17	12.1	288	38	12.5
Tendon and waste	66	8	3.0	66	5	2.9
Half loin	1241	71		1262	233	
Fatty tissue	315	89	25.4	260	129	20.6
Muscular tissue	719	93	57.9	775	113	61.4
Bone	120	45	9.7	144	48	11.4
Tendon and waste	70	10	5.6	65	13	5.2
9-10-11 rib cut	561	112		604	112	
Fatty tissue	177	75	31.6	144	83	23.8
Muscular tissue	272	70	48.5	330	29	54.6 n.s.
Bone	75	6	13.4	87	6	14.4 n.s.
Tendon and waste	39	7	7.0	42	7	7.0

It will be seen in Table 1 that the Cheviot carcasses contained significantly more protein* and water than the Romney carcasses. Related to these findings were the heavier weight and higher percentage of muscular tissue in each of the joints dissected from Cheviot carcasses (Table 2) although in no cases were the differences between the two breeds statistically significant. The Cheviots also had a higher dressing percentage (Table 1). The absolute and proportionate weight of both chemical fat and dissectible fatty tissue was however slightly greater in the Romney carcasses. None of these differences was statistically significant.

Discussion

Although these results are based on a small number of observations they show that the Cheviot carcasses contained more protein and water. These findings together with the dissection results, strongly suggest that the Cheviots studied were more muscular animals than the Romneys. Previous comparisons (Hirzel 1939; Walker 1949) based on measurements, grade, and dissection of the carcasses, have indicated that the immature Cheviot was likely to be a less fat animal than the Romney at the same age. Similarly, von Borstel (1952) and von Borstel and Barton (1952) showed from the dissection of the shoulder region of 6 Romney and 6 Cheviot mature ewes of comparable live weight that the Cheviots had 10% more muscular tissue than the Romneys but the latter had 10.9% more fatty tissue. The difference in weight and proportion of muscular tissue was not found to be statistically significant but the heavier and proportionally greater amount of fatty tissue in the shoulder region of the Romneys differed significantly from the Cheviots.

References

Barnicoat, C. R.; Shorland, F. B. 1952: *N.Z. J. Sci. Tech. A33 (5):* 16.
Barton, R. A.; Kirton, A. H. 1958: *N.Z. J. agric. Res. 1:* 783.
Hirzel, R. 1939: *Onderstepoort J. vet. Sci. 12:* 379.
Kirton, A. H. 1957: *M.Agr.Sc. Thesis* (Lodged in Massey Agricultural College Library).
Kirton, A. H.; Barton, R. A. 1958: *J. agric. Sci. 51:* 265.
von Borstel, F. 1952: *M.Agr. Sc. Thesis* (Lodged in Massey Agricultural College Library).
von Borstel, F.; Barton, R. A. 1952: *Sheepfmg Annu. Massey Agric. Coll.*, p. 49.
Walker, D. E. 1949: *N.Z. J. Agric. 79:* 219.

* Protein = dried, fat-extracted, and ash-free residue (c.f. Barnicoat and Shorland 1952).

MINERALISATION OF NITROGEN AND SULPHUR IN SULPHUR-DEFICIENT SOILS

By J. G. White, *Canterbury Agricultural College

(Received for publication, 1 September 1958)

Summary

Incubation of sulphur-deficient soils, after liming those of low pH, showed that lime increased both mineral nitrogen and sulphate sulphur formed over a given period, compared with unlimed soil. The total N : S ratio in neutral soils was 7 or 8 : 1, while mineral N and sulphate S accumulated in the ratio of about 10 : 1. In acid soils, these ratios were considerably higher. It is suggested that mineralisation and immobilisation of sulphur in the soil may follow a similar pattern to that of nitrogen.

Introduction

Sulphur deficiency has recently been noted in a number of soils in New Zealand, especially in the South Island. The main responses to sulphur have been on pasture legumes, although lucerne and rape have also given increased growth (Lobb 1952). In these deficient areas, soil analyses have shown no close correlation between the total soil sulphur and sulphur deficiency (Walker *et al.* 1955). In grassland soils the main reservoir of both soil sulphur and soil nitrogen is in the organic matter (Walker 1956, 1957) and both are being slowly mineralised; the sulphur to soluble sulphate, and the nitrogen to ammonia and nitrates, forms in which they are taken up by plants, or leached from the soil. According to Walker *et al.* (1956), in a grass and clover pasture the grasses utilise almost all the mineralised nitrogen and sulphur, and legumes are therefore dependent for sulphur on outside sources such as atmospheric returns or weathering of sulphur-containing minerals, if they are to "fix" nitrogen. Therefore, sulphur responses on pasture legumes in many South Island areas may be due to low atmospheric returns of the nutrient.

In this investigation, an incubation trial was carried out to study the mineralisation of nitrogen and sulphur in the soil, especially where lime had been added to soils of low pH.

Soils and Experimental Methods

Soils

The "A" horizons of ten soils were collected from various parts of Canterbury and north Otago, all but two being known to respond to sulphur application in the field. Four of the soils belonged to the Waiareka complex series from near Oamaru, where Lobb (1952) had obtained marked responses to sulphur application on clover and rape. Four more soils were obtained from the Canterbury foothills on soil types Cass fine sandy loam (Lake Coleridge), Kowai fine sandy loam (Windwhistle), Hurunui silt loam—gleyed associate (Lee's Valley), and Oxford silt loam (Oxford). Sulphur responses on clovers had been

* Postal address: Lincoln College, Christchurch, N.Z.

obtained on all these soils by Walker *et al.* (1955). The final two soils, from Lincoln, were a Temuka heavy silt loam and a Templeton silt loam, neither known to be sulphur deficient.

Experimental Methods

The soils were analysed for pH, organic carbon (Allison 1935), total nitrogen, and total sulphur (Swanson and Latshaw 1922).

Duplicate samples of each soil (100 g) were kept at 50% of water-holding capacity and incubated at 30°C for 28 days. The four soils with pH below 6 received a dressing of $Ca(OH)_2$ equal to 5.4 tons of $CaCO_3$ per acre before incubation. The soils were then analysed for ammonia, nitrate, and sulphate. Marsden and Pollard's (1937) benzidine method

TABLE 1. Results of Analyses

Soil Analyses

Soil	pH	%C	%N	%S	N : S
Waiareka 1	7.2	4.61	.37	.056	6.6
Waiareka 2	6.8	3.46	.41	.034	11.9
Waiareka 3	6.7	3.30	.30	.042	7.1
Waiareka 4	6.3	3.55	.33	.045	7.3
Temuka	7.2	5.74	.46	.072	6.3
Templeton	6.0	3.99	.30	.036	8.3
Cass	5.3	4.79	.31	.032	9.8
,, (limed)					
Kowai	5.3	3.55	.30	.034	8.7
,, (limed)					
Hurunui	5.3	3.39	.24	.017	13.9
,, (limed)					
Oxford	5.3	4.65	.35	.033	10.6
,, (limed)					

Incubation Analyses

Soil	NH_4-N p.p.m.	NO_3-N p.p.m.	SO_4-S p.p.m.	Mineral N : S
Waiareka 1	tr.	86	8.1	10.6
Waiareka 2	tr.	96	9.4	10.3
Waiareka 3	tr.	113	6.0	18.8
Waiareka 4	tr.	91	7.3	12.4
Temuka	4	173	17.8	9.9
Templeton	2	93	8.5	11.3
Cass	54	25	3.6	21.9
,, (limed)	113	25	10.0	13.8
Kowai	81	59	6.3	22.2
,, (limed)	9	162	5.5	31.1
Hurunui	117	4	6.7	18.1
,, (limed)	163	8	6.5	26.3
Oxford	127	25	4.1	37.0
,, (limed)	116	109	11.0	20.5

was used for micro-determination of the sulphate after it had been extracted by shaking 40 g of soil with 250 ml of 0.001 N HCl for two hours.

RESULTS

The analytical data are presented in Table 1.

The total N:S ratio in neutral soils averaged slightly above 7, but in acid soils, with higher C:N and C:S ratios, the total N:S ratios were between 9 and 14.

Upon incubation, mineral nitrogen accumulated in different forms according to pH. Where the soil pH was high, mineral nitrogen accumulated mainly as nitrate, but in the more acid Canterbury hill soils, ammonia was the dominant form. Liming the latter soils increased the total mineral nitrogen which accumulated mainly as nitrate in two soils, and mainly as ammonia in the other two soils.

The soluble-sulphate content was increased significantly by liming in the Cass and Oxford soils, but little change occurred in the Kowai and Hurunui soils, possibly through lack of suitable organisms to mineralise organic sulphur.

Not all of the sulphate may have been extracted from the soil by the method used, as, according to Ensminger (1954), sulphate, although available to plants, may be absorbed by mineral colloids and in this state is not readily displaced by chloride in the extracting solution used in this experiment.

DISCUSSION

It is generally accepted that the plant takes up soil sulphur in the form of soluble sulphate. The rate of mineralisation of the organic sulphur to sulphate therefore assumes special significance, especially on sulphur-deficient soils, as the organic sulphur is the main source of sulphate in the soil, atmospheric returns being very low in many inland areas of the South Island (Walker *et al.* 1956).

Lime effected large increases in the sulphate content of the Cass and Oxford soils, supporting the work of Wiklander *et al.* (1951) and Ellet and Hill (1929) who also obtained increases in soil sulphate after applying lime. There is a distinct similarity here to the relatively well known pattern of nitrogen mineralisation, which is also increased by liming.

The total N:S ratio of 7 or 8 to 1 for the neutral soils seems normal figures, as Donald and Williams (1954), working on some Australian grassland soils, found a mean N:S ratio of 7.34:1 with a high correlation between total soil sulphur and nitrogen. Walker and Adams (1958) reached similar conclusions for a number of N.Z. soils (mean N:S ratio 7.64:1). Higher ratios are to be expected on more acid soils where the C:N and C:S ratios are also high.

Mineral N : S ratios (NH_4-N + NO_3-N : SO_4-S) were about 10 : 1 for the neutral soils, but the ratio in acid soils after incubation varied between 20 : 1 and 40 :1. Liming these acid soils before incubation did

not affect the ratio appreciably, as in most cases mineral nitrogen and sulphur both increased. In soils where total N : S ratios are normal (7 : 1 to 8 : 1), the relative rates of mineralisation of nitrogen and sulphur would appear to be very similar, producing mineral nitrogen and sulphate sulphur in the proportion of about 10 : 1.

The acceleration of soluble-sulphate formation may be yet another of the functions of lime in the soil. The amount of sulphate formed from the organic matter must clearly be related to the total sulphur contained there, and will be influenced by the C : S ratio, pH, and other factors in much the same way as they influence the formation of mineral nitrogen. Further work on this subject may be of considerable interest, especially the study of mineralisation of nitrogen and sulphur at different pH and temperature levels. There may be a continuous cycle of mineralisation and immobilisation as with nitrogen (Hiltbold *et al.* 1950) the nett amount of mineral surphur formed depending on the results of these opposing biological processes.

Acknowledgements

This work was carried out as part of the requirements for a M.Agr.Sc. degree. I wish to thank Professor T. W. Walker and other members of the Soils Department at Lincoln College for the considerable help given to me in this work.

References

Allison, L. E. 1935: *Soil Sci. 40:* 311-20.
Donald, C. M.; Williams, C. H. 1954: *Aust. J. agric. Res. 5:* 664-88.
Ellet, W. B.; Hill, H. H. 1929: *J. agric. Res. 38:* 697-711.
Ensminger, L. E. 1954: *Proc. Soil Sci. Soc. Amer. 18:* 259-64.
Hiltbold, A. E.; Bartholomew, W. V.; Werkman, C. H. 1950: *Ibid. 15:* 166-73.
Lobb, W. R. 1952: *N.Z. J. Agric. 89:* 434-8.
Marsden, A.; Pollard, L. 1937: *Soc. Chem. Ind. 56:* 464 T.
Swanson, C. O.; Latshaw, W. L. 1922: *Soil Sci. 14:* 421.
Walker, T. W. 1956: *J. Sci. Fd Agric. 7:* 67-72.
———1957: *J. Brit. Grassl. Soc. 12:* 10-18.
Walker, T. W.; Adams, A. F. R. 1958: *Soil Sci. 85:* 307-18.
Walker, T. W.; Adams, A. F. R.; Orchiston, H. D. 1955: *N.Z. J. Sci. Tech. A36:* 470-82.
———1956: *Plant and Soil 7:* 290-300.
White, J. G. 1955: *Thesis presented for M.Agr.Sc.* (Lodged in Canterbury Agric. Coll. Library.)
Wiklander, L.; Hallgren, G.; Jonnson, E. 1951: *Soils & Ferts. 14:* 208.

HOUSING A RABBIT COLONY

By I. J. Cunningham, Animal Research Station, Department of Agriculture, Wallaceville

(*Received for publication, 7 October 1958*)

Summary

A description is given of cages and their arrangement for efficient management of a rabbit colony.

Introduction

It is desirable to keep laboratory rabbits on wire floors to avoid losses from coccidiosis in the young. The housing system should also provide a firm nest for the litter at birth and for its first two weeks of life and should ensure quiet and privacy for the doe and her young over this period. A method to do this with moderate capital outlay for equipment and which makes an effective use of space and labour, has been employed at Wallaceville and is described below.

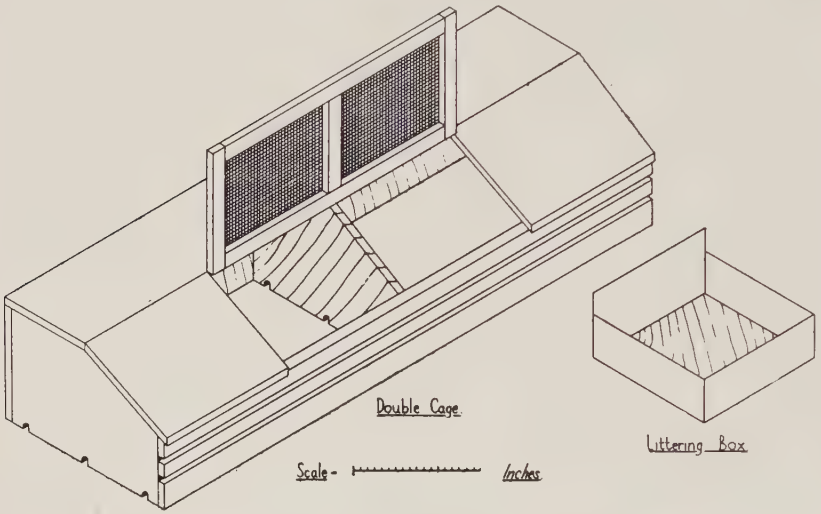
The Cage

The cage is illustrated in Fig. 1 which also shows a drawing to scale.

The outside measurements are the same as for the guinea-pig cages used at Wallaceville, which standardises construction and makes interconversion possible, and the rabbit cage is supported in the same way on three bars made of $\frac{1}{2}$-in. galvanised-iron water pipe. Each cage is divided entirely into two separate compartments 3 ft $2\frac{1}{2}$ in long by 2 ft wide; resting on the galvanised iron bars is a wire-mesh floor made of crimped 14-gauge wire woven so as to leave spaces of $\frac{1}{2}$ inch by $\frac{1}{2}$ inch. This mesh is wider than the one used for guinea pigs because of the larger size of faecal pellets in rabbits. The outer half of each compartment is covered by a fixed solid top and a single door closes the inner halves of each compartment. Such a single door opening on two compartments at once has not been found in practice to give rise to any problems. The rabbits tend to gnaw at the wooden bars of the cages for a while when they first go in but soon give up the practice.

Cage Arrangement

Cages are arranged exactly the same way as guinea-pig cages in two tiers with the wire floor of the bottom tier 20 inches above the floor of the building, and the wire floor of the top tier 30 inches higher. Eight inches below the top tier is a galvanised-iron tray covered with paper to catch waste matter: waste from the bottom tier of cages falls to the concrete floor of the building which is covered below the cages with cheap paper, to make cleaning easier and quicker. Over the paper beneath both tiers is spread a thin layer of sawdust containing 5% of "straight" superphosphate which is the cheapest available source of weak acid to absorb ammonia and eliminate smell from this source.



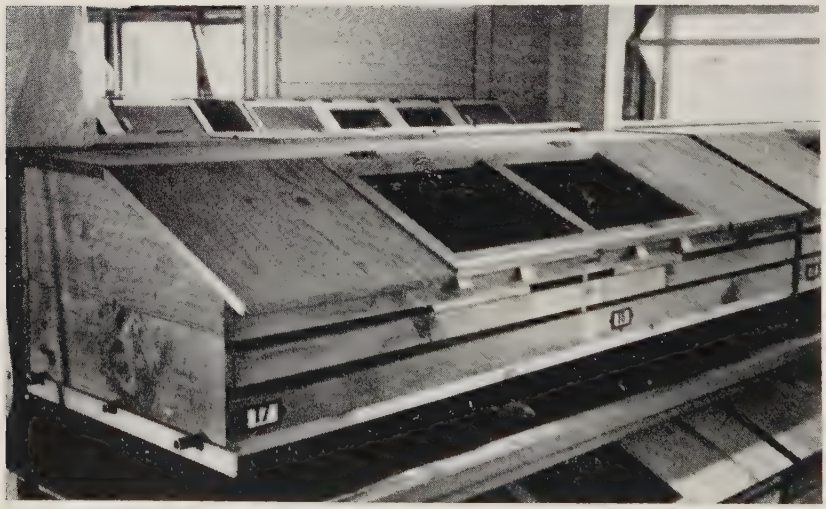


Fig. 1.—Scale drawing and photograph of rabbit cage.

Superphosphate used in this way need not be wasted because the cleanings can be employed as a fertiliser for the green-feed garden.

Food Hopper and Water

The three-compartment food hopper used for guinea pigs (Cunningham 1958) has been found satisfactory for rabbits, and pellets, chaffed hay, and green grass, when available, are fed in separate com-

partments. Water is supplied in an open-top metal or glass jar which is replenished, as necessary, through a valve at the end of a flexible hose.

Cleaning

The trays and floors are cleaned three times a week. It has been found that the wire-mesh floors of the cages must be cleaned once a week. The whole cage is dismantled and the cage and supporting bars cleaned about once in three months.

Accommodation for Breeding

One compartment is allowed for a breeding doe. When she is ready to make her nest a littering box containing some hay is placed in the cage. The littering box (see Fig. 1) is 18 inches square, has 3 sides 5 inches high and one side 8 inches high and has a floor of hardboard. The box is put in the compartment under the solid fixed top and the 8-inch side faces the front. The doe thus has an enclosed and private refuge in which she can build her nest and rear the litter. The littering box is removed as soon as the young start to move out of it into the rest of the compartment, which occurs when the litter is 10 days or a little older. If this is done, the floor of the box keeps dry: if the box is left longer, the floor and the bedding may become wet. The young and the doe live satisfactorily until weaning time on the wire mesh with no bedding.

Carrying Capacity

Each breeding buck is allotted one compartment and, similarly, a breeding doe has one compartment. After weaning, four rabbits of one sex are kept in one compartment.

A room 30 by 50 ft would lend itself to an arrangement of a central corridor with cages on either side. Such a room would accommodate 100 cages which would provide 200 compartments in which could be kept 90 breeding does, 10 bucks, and 400 non-breeding adult rabbits. or, of course, any other combination found desirable.

ACKNOWLEDGEMENT

Acknowledgement is made to Mr. A. L. Bryant for the drawing and photographs in Fig. 1.

REFERENCES

Cunningham, I. J. 1958: *N.Z. J. agric. Res. 1:* 750-6.

TRIALS OF PLASTIC LINING IN GLASSHOUSES

By S. Challenger, Canterbury Agricultural College*

(*Received for publication, 16 September 1958*)

Summary

Trials with plastic lining in glasshouses have resulted in slight improvement in minimum temperatures, and in early yields of tomatoes planted in the colder months. No result in total cropping in tomatoes was obtained.

Introduction

Trials in other countries have given worth-while results from the use of a plastic lining in glasshouses, showing considerable economy in fuel (N.I.A.E. 1956), and, in the case of tomatoes, faster growth and earlier cropping. Trials were carried out at Lincoln to see whether these results could be duplicated in Canterbury.

Method

The glasshouse chosen for the trial had been used for growing tomatoes for several years and was of a design normally used in Canterbury for this purpose (Coombe *et al.* 1957). The house was heated by pyrotenax cable attached to the side walls just above the bottom plate. Heating was thermostatically controlled, the thermostat being situated in the south end of the house. For the purpose of the trial the house was divided into two halves by the use of a partition, one end of the house being lined with plastic whilst the other end was left unlined. The plastic lining, 0.0015 inch thick, was attached to the wooden framework of the house by staples, using a tacking gun. The lining was tacked to the side walls and half way up the roof bars. The partition and house end were covered from floor to ridge, except for a doorway space, six by three feet, in the partition.

The first crop, of Potentate, was planted 28 August 1957 and removed 5 February 1958, and for this crop the south end was lined with plastic. The second crop, of Moneymaker, was planted 7 February 1958 and removed 9 July 1958 and for this crop the north end was plastic lined. Temperature records were kept from 15 September 1957 to the end of the experiment, maximum and minimum temperatures for the previous 24 hours being recorded for both ends of the house. Yields of fruit for both ends were also recorded, the house being cleared completely of ripe fruit on each occasion that picking was carried out.

Results

The temperature records are summarised in Table 1. Daily records were averaged at the end of the week, and these figures used. This method was used to compress the data into digestible form, and show the general temperature trend without daily variation.

* Postal address: Lincoln College, Christchurch, New Zealand.

TABLE 1. WEEKLY AVERAGES OF TEMPERATURES IN NORTH AND SOUTH ENDS OF TOMATO HOUSE

Date	Maximum		Minimum		Difference Max.-Min.	
Week Ending	North	South	North	South	North	South
Crop 1. South end, plastic-lined						
22/ 9/57	82.8	82.8	52.3	53.3	30.5	29.5
29/ 9/57	82.7	81.7	52.6	52.8	30.1	28.9
6/10/57	84.0	84.6	52.0	52.7	32.0	31.9
13/10/57	87.1	85.8	53.0	53.7	34.1	32.1
20/10/57	86.4	87.0	53.8	54.1	32.6	32.9
27/10/57	90.0	88.1	53.7	54.1	36.3	34.0
3/11/57	89.3	87.8	54.2	55.5	35.1	32.3
10/11/57	87.3	86.3	55.6	53.3	31.7	33.0
17/11/57	90.1	88.0	55.3	55.3	34.8	32.7
24/11/57	90.8	88.3	56.6	57.2	34.2	31.1
1/12/57	82.1	81.1	55.6	57.0	26.5	24.1
8/12/57	88.8	84.7	55.1	55.1	33.7	26.9
15/12/57	86.7	86.3	58.8	59.4	27.9	29.6
22/12/57	87.1	86.3	55.6	55.4	31.5	30.9
29/12/57	89.8	87.8	52.6	54.2	37.2	32.6
5/ 1/58	93.4	92.8	52.8	52.8	40.6	40.0
12/ 1/58	88.7	84.6	54.7	54.0	34.0	30.6
19/ 1/58	92.1	89.6	59.1	59.7	33.0	29.9
26/ 1/58	98.6	96.8	59.6	59.4	39.0	37.4
2/ 2/58	94.4	93.1	62.3	63.0	32.1	30.1
9/ 2/58	96.0	93.8	64.5	65.0	31.5	28.8
Crop 2. North end, plastic-lined						
16/2/58	81.6	76.6	61.3	61.3	20.3	15.3
23/2/58	80.4	79.6	57.4	58.0	23.0	21.6
2/3/58	90.6	88.1	57.7	55.1	32.9	33.0
9/3/58	90.0	87.4	58.7	58.8	31.3	28.6
16/3/58	80.7	77.3	57.3	56.3	23.4	21.0
23/3/58	90.6	87.1	53.6	53.6	37.0	33.5
30/3/58	80.8	81.0	55.0	54.3	25.8	26.7
6/4/58	85.3	84.0	52.1	52.8	33.2	31.2
13/4/58	81.7	80.0	53.0	53.4	28.7	26.6
20/4/58	76.0	73.6	51.7	54.0	24.3	19.6
27/4/58	76.8	74.1	53.8	54.7	23.0	19.4
4/5/58	79.8	77.8	58.0	58.6	21.8	19.2
11/5/58	76.7	76.0	58.0	57.7	18.7	18.3
18/5/58	72.7	74.6	53.4	50.8	19.3	23.8
25/5/58	73.1	74.0	50.8	51.3	22.3	22.7
1/6/58	81.3	81.6	52.1	52.6	19.2	19.0
8/6/58	77.4	78.0	56.3	55.8	21.1	22.2
15/6/58	79.6	79.3	54.6	54.3	25.0	25.0
22/6/58	78.0	77.8	52.3	52.6	25.7	25.2
29/6/58	78.1	78.1	55.7	52.7	22.4	25.4
6/7/58	80.1	79.8	49.8	49.3	30.3	30.5
13/7/58	80.0	79.6	52.5	52.5	27.5	27.1

The cropping records are summarised in Table 2. The grand total was broken up into monthly or fortnightly totals to show up any tendency to early cropping. In both crops the original total planting was 569, 293 in the north end and 276 in the south. Some plants were removed during the season with disease and the result is therefore calculated on a basis of yield per plant and not total yield. In Crop 1 the exact numbers of plants removed were not kept, but a reasonably accurate estimate was used for calculating yield per plant. The plants in Crop 2 were stopped before full growth to enable preparation for the succeeding crop to be carried out.

TABLE 2. TOMATO YIELDS IN NORTH AND SOUTH ENDS OF HOUSE

Crop 1. (28 Aug. 1957 to 5 Feb. 1958)

South end, plastic-lined

Potentate

Month	North (273 plants)*				South (246 plants)*			
	Total Yield		Yield/Plant	Incr.	Total Yield		Yield/Plant	Incr.
	lb	oz	lb oz	%	lb	oz	lb oz	%
Nov.	21	8	1.3	—	49	8	3.2	155
Dec. 1-15	297	8	1 1.4	—	296	8	1 3.3	11
Dec. 16-31	762		2 12.7	74	395	8	1 9.7	—
Jan.	785	8	2 14	132	304	9	1 3.8	—
Feb.	83		4.9	7	70		4.6	—
Total	1949	8	7 2.3	57	1116	1	4 8.6	—

Crop 2. (7 Feb. 1958 to 9 July 1958)

North end, plastic-lined

Moneymaker

Month	North (293 plants)				South (266 plants)			
	Total Yield		Yield/Plant	Incr.	Total Yield		Yield/Plant	Incr.
	lb	oz	lb oz	%	lb	oz	lb oz	%
May	315	8	1 1.2	46	195	9	11.8	—
June	485	5	1 10.5	55	284	4	1 1.1	—
July	43		2.4	37	29	12	1.7	—
Total	843	13	2 14.1	50	509	9	1 14.6	—

* Estimate—see text.

DISCUSSION AND CONCLUSIONS

Temperature results through both crops show a gradient from north to south, the north end having the highest maximum and lowest

minimum. This has a confusing effect in examining the results. However, there does appear to be a small advantage in heat retention under the plastic liner. During Crop 1 the north (unlined) end showed minimum readings 0.5° to 1.0°F lower than the lined end on 14 weeks out of 21 (with three equal). During Crop 2, although the south (unlined) end had a higher minimum temperature on 10 weeks out of 22 (with three equal), the mean of the temperature differences showed an average lower temperature in the south end. In this case the use of the plastic was able to reverse the natural temperature gradient within the house when changed from end to end. The use of plastic had very little effect upon the maximum figures reached. During Crop 1 the north (unlined) end showed maximum readings consistently 1° to 2°F higher than the south end, but during Crop 2 the north (lined) end still showed maximum readings higher than the south end. There is a possible slight reduction of maximum temperatures under plastic, but this conclusion is not a very certain one. During Crop 1 the north (unlined) end had the higher temperature on 18 weeks out of 21 (with one equal), whilst during Crop 2 the north (lined) end had the higher temperature on 16 weeks only, out of 22 (with one equal), showing a possible ability only to reverse the natural temperature gradient.

Yield results are dominated by a difference in cropping capacity between the north and south ends, which is similar in both Crop 1 and 2, the north end showing a 50% advantage over the south end. In Crop 1 however, the earliest yield came from the south (lined) end, during the first three weeks of cropping, but this result was not repeated in Crop 2, which suggests that plastic lining would be of greater value only with early crops of tomatoes planted during the colder months of the year, rather than with summer-planted crops. The changing of plastic between ends did not influence total yield significantly and no correlation between temperature records and yields could be traced.

No difference in plant growth between the two ends could be observed until late in the season. The extreme south end in both crops was the poorest in growth.

These results are rather disappointing in view of the excellent results given by plastic lining elsewhere in New Zealand (Hockey 1956) and it is difficult to account for them. The house itself, having both a fertility and temperature gradient, previously unappreciated, was not ideal, and it is possible that the lining was not of sufficient extent to produce an effective result. Overseas reports (Spice 1956) mentioned the retention of water vapour within the plastic lining and this was the main reason for not lining the roof completely. However, it appears that minimum temperatures were improved, as well as early yield from tomatoes planted in the colder months, even if no result in total cropping was obtained.

REFERENCES

Coombe, J.; Nottage, I. L.; Richardson, C. S.; Gilbert, S. O. 1957: *N.Z. Dep. Agric. Bull. 373.* p. 4.
Hockey, K. C.; Richards, M. 1956: *Fruit & Prod. Oci. 1956.* pp. 14-15.
National Institute of Agricultural Engineering 1956: *Test Report 125.*
Spice, H. R. 1956: *Grower 45:* 637.

PHOTOSENSITIVITY DISEASES IN NEW ZEALAND

XV. A CHEMICAL PROCEDURE FOR THE DETECTION OF FACIAL ECZEMA TOXICITY IN PASTURE

By D. D. Perrin*, Ruakura Animal Research Station, Department of Agriculture, Hamilton

(*Received for publication, 7 August 1958*)

Summary

An empirical chemical method for the detection of facial eczema toxicity in pasture is described. Most pasture samples collected from 7 areas over 1–3 autumns showed good correlation between this test and toxicity as assessed by guinea-pig feeding. The substance which is the basis of the test is not the facial eczema poison. Samples from 6 other areas which did not produce toxic pasture did not give positive chemical tests. Of 35 pure species of plants similarly tested only *Mentha* and related species, especially pennyroyal, gave spurious chemical tests.

Introduction

The use of the guinea pig in facial-eczema research (Perrin 1957) has enabled considerable progress to be made towards the isolation and chemical characterisation of the poison responsible for this disease (White 1958). However, in its present form the guinea-pig test requires a minimum of 3–5 weeks' feeding before unequivocal evidence of facial-eczema toxicity of pasture can be obtained.

For many purposes, and particularly for the study of field aspects of the disease, a much more rapid indication of toxicity is required. Such an indication would, by greatly increasing the chances of selecting toxic areas, considerably facilitate the collection of toxic pasture, would enable such pasture to be examined while still actually growing, and would at the same time permit much more direct investigation of the effects on stock of various types of management.

In an endeavour to obtain a chemical method for this purpose, detailed comparisons were made of ultraviolet and visible absorption spectra for toxic and non-toxic extracts at each stage of the basic extraction procedure described by White (1958). To date, although a concentration of the poison of about 20,000 times its level in dried grass has been reached (White 1958), no significant differences in absorption spectra between toxic and non-toxic extracts have been observed.

However, during this work, fractionation of extracts on columns of activated alumina showed the presence of traces of a white material in the small number of toxic samples examined. This material was not present in the non-toxic samples similarly treated. Subsequently it was found that by using less-active alumina, followed by passage through a charcoal column, more rigid standardisation of conditions for detect-

* Present address: The John Curtin School of Medical Research, The Australian National University, Canberra, Australia.

ing this white material was possible. A method based on the presence or absence of this white material has been developed and appears to distinguish qualitatively between normal and facial-eczema-producing pastures.

Briefly, this method, which is known as the "Beaker Test", consists of ether extraction of dried pasture, followed by successive evaporation and fractionation, the final extract being evaporated in a beaker in a draught of air. The white material, if present, appears as a thin opaque film on the wall of the beaker as evaporation proceeds. By adapting the original procedure so that soxhlet thimbles could be used, it was possible for batteries of samples to be tested, one operator and assistant being able to complete 24 tests in two working days. These tests, on 50 g dried pasture, were used routinely during autumn 1957 in conjunction with grass collection for guinea-pig testing. The experimental procedure set out below was somewhat arbitrarily selected as providing a high degree of reproducibility without requiring a very careful standardisation of operating conditions.

METHOD

A sample of pasture dried at 90–100°C in a coke-fired tray grass drier is ground in a Wiley mill to pass a 1 mm screen. A 50 g portion is extracted in a 41 × 123 mm soxhlet thimble, using ethyl ether, for 5 hours. The ether extract is evaporated on to 2 g Hyflo-Super-Cel filter aid, in a stream of air, and the residue is vacuum desiccated over concentrated sulphuric acid.

The dried material is extracted with 4–5 successive portions of methanol to give a final volume of filtrate of 25 ml. Following the addition of 6 ml water the extract is shaken with 3 successive 25-ml volumes of petroleum ether (40/60°C), the petroleum ether phases being discarded. The aqueous methanol solution is evaporated in a draught of air on a boiling water-bath, and the residue is dried in a vacuum desiccator. The dried material is taken up in a small volume of ethyl ether : acetone (1 : 1) and transferred to an alumina column, 100 × 13 mm, prepared by adding activity I alumina to ether containing 2% glacial acetic acid and then washing well with ether. The chromatogram is developed and eluted with ether : acetone, the eluate being collected until after the main yellow band has been carried through.

Following evaporation in a draught of air on a boiling water-bath, and vacuum desiccation, the residue is dissolved in methanol containing 1% benzene and transferred to a charcoal column, 100 × 9 mm, prepared in methanol from Darco G60 : Hyflo-Super-Cel (1 : 4), dried at 120°C. A 3-ft pressure head of solvent is applied. The first 25 ml of eluate is collected and evaporated in an air draught, using a bench fan, at room temperature, in a 50-ml beaker.

The extent and intensity of any white deposit formed on the wall of the beaker is assessed visually by comparison against similar deposits from aliquot portions of selected dried pasture samples. These standards are stable for several weeks but must then be renewed. The appearance of these standards is approximated by evaporating under

similar conditions solutions of phenolphthalein in methanol, the deposits obtained from 0.5 and 1.0 mg phenolphthalein corresponding to Beaker Test Levels of "1" and "2", respectively, while "3" is defined as any appreciably higher level.

Results and Discussion

The results obtained to date by applying the Beaker Test in this form to 266 dried pasture samples may be summarised as follows:

Guinea-pig Facial-eczema Liver Damage

Beaker Test	None	Mild	Mild to Mod.	Mod.	Mod. Sev. or Sev.
0	150	6	5	4	1
1	7	3	0	3	3
2	14	5	2	6	17
3	10	2	1	5	22

These results include samples obtained from Claudelands Showgrounds, Hamilton (3 years); Ruakura Animal Research Station (2 years); Manutuke Research Station, Gisborne (2 years); Makaraka Racecourse, Gisborne; 3 farms near Gisborne; and 5 farms in the Waikato.

The guinea-pig liver damage was assessed under standard conditions as previously described (Perrin 1957). All results given above were for one guinea pig and one beaker test per sample, as being typical of the conditions under which the test would ordinarily be applied. Particularly at lower levels of toxicity, the response of the guinea pig to the facial-eczema poison is somewhat variable (Perrin 1957), while repeated determinations on individual pasture samples indicate an uncertainty in the beaker test of about $\pm$ 1 unit. However, a strong correlation has been found between the two tests. Thus 56% of all samples tested have been negative by both tests, while of 43 samples giving moderately severe or severe liver damage only 1 failed to give a positive beaker test and all but 4 gave beaker tests of "2" or "3". Similarly, of 40 samples giving a beaker test of "3", 22 produced moderately severe or severe, and 5 moderate, liver damage. For beaker test "2", 23 out of 44 samples belonged to these two groups.

A delay of several months commonly occurred between the collection and beaker testing of pasture samples and their feeding to guinea pigs. It is possible that some of the samples in the groups giving beaker tests "2" and "3", guinea pig normal, may have been toxic at the time of collection and have diminished in toxicity in the period intervening between the two tests.

Guinea-pig testing is not yet complete for all areas and the present results probably do not give a complete picture of the occurrence of toxicity during autumn 1957. However, to examine more closely the application of the beaker test to individual areas, results at present available are presented in Fig. 1 on an area basis. The ordinates indicate the levels of beaker test and of liver damage obtained. Classi-

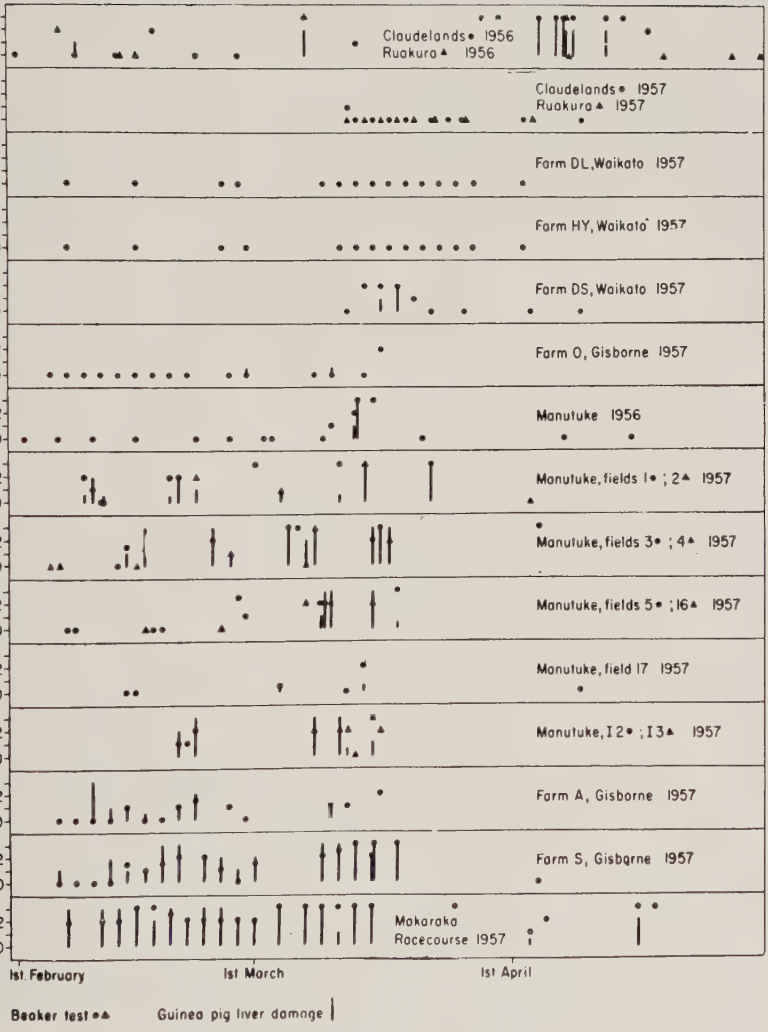


Fig. 1.—Results on area basis.

fication of the liver damage was as follows: 0, normal; 1, mild or mild-to-moderate; 2, moderate: 3, moderately severe or severe. Additional to the data of Fig. 1, 2 areas where no toxic grass was collected gave, in 16 beaker tests, only 1 of level "1" and none of levels "2" or "3".

Two sources of interfering substances were encountered. A clover-rich, spring pasture sample contained appreciable amounts of a material identified as the oestrogenic isoflavone, formononetin (E.P. White, pers. comm.) and confirmed by quantitative ultraviolet-absorption measurements. This substance, which is present in some clovers under certain conditions, is believed to be an unlikely source of interference in autumn pasture. Its appearance is somewhat different from the material of the

beaker test, from which it may be easily distinguished by its blue fluorescence in alkaline solutions in ultraviolet light.

The other, more important, interference arose from the presence in some pasture samples of significant concentrations of certain plant species, especially pennyroyal (*Mentha pulegium*). In experiments using samples, collected in late summer and autumn, of pure species, which may sometimes occur in pastures, no positive beaker tests were obtained from yarrow (*Achillea millefolium*), browntop (*Agrostis tenuis*), sweet vernal (*Anthoxanthum odoratum*), prairie grass (*Bromus catharticus*), Californian thistle (*Cirsium arvense*), Scotch thistle (*C. lanceolatum*), hawkweed (*Crepis capillaris*), Bermuda grass (*Cynodon dactylon*), cocksfoot (*Dactylis glomerata*), tall fescue (*Festuca arundinacea*), *Gnaphalium purpureum,* Yorkshire fog (*Holcus lanatus*), barley grass (*Hordeum murinum*), toadrush (*Juncus bufonius*), *Lotus uliginosus, Panicum miliaceum, Paspalum dilatatum,* plantains (*Plantago lanceolata, P. major*), *Poa annua,* buttercup (*Ranunculus sardous*), docks (*Rumex crispus, R. obtusifolius*), ratstail (*Sporobolus capensis*), *Stachys arvensis,* suckling clover (*Trifolium dubium*), alsike (*T. hybridum*), red clover (*T. pratense*), white clover (*T. repens*), or subterranean clover (*T. subterraneum*). Positive tests were obtained from pennyroyal, self-heal (*Prunella vulgaris*), and to lesser extent from common mint (*Mentha viridis*) peppermint (*M. piperita*), and spearmint (*M. spicata*). Interference from pennyroyal was much more pronounced than from any other species tested, repeated determinations with samples of pennyroyal from different areas indicating that 1% contamination of a pasture sample with this weed would give a false positive beaker test. Although the material from pennyroyal appears to exhibit a slightly different melting behaviour from that of white material of the beaker test (E. P. White, pers. comm.), no satisfactory method has been so far devised for distinguishing the two materials. In the meantime, although contamination of autumn pastures by pennyroyal is not very common, it is necessary before applying the beaker test to ascertain that this weed is not present in the sample.

Extraction work has shown that the white material which forms the basis of the test described above is not the poison responsible for the liver damage in facial eczema (E. P. White, pers. comm.). Also, it is considered unlikely, from its known properties, that the facial-eczema poison would be stable to certain of the steps used routinely in this test. The relationship of the beaker-test material to the facial-eczema poison is of some interest, especially as results in Fig. 1 show that both substances generally rise and fall in pasture together. It is possible that the former is a degradation product of the latter, or at least a product of similar biochemical processes. More detailed study of the beaker-test material in fresh as well as in dried pasture may contribute to the knowledge of the plant physiology involved in the elaboration of the poison.

The total fraction eluted from the charcoal column is of the order of 0.02% to 0.05% of the original dried pasture. Observations suggest that the white material is only a very small fraction of this eluate.

A crude preparation of the white material has been examined (White 1958). Subsequent work has also failed to reveal any reliable chemical differences between it and the water-clear residue obtained in non-toxic samples. The material is combustible without residue and, hence, is presumably organic. However, in the absence of any characteristic ultraviolet-absorption spectrum or any selective chemical reaction, no indication as to the nature of the deposit can be given.

The original beaker tests were carried out using 200-g samples and extraction procedures more closely related, especially as regards evaporation, to the basic extraction process for the facial eczema poison (White 1958). Forty samples, 14 of which were toxic to guinea pigs, were assessed by beaker tests using the earlier 200 g and the subsequent 50 g procedures. Good agreement between the two series was obtained.

In an earlier experiment a sample of toxic dried pasture was hand-picked into two fractions; one of these was the grass growing at the time the sample was cut, the other was mainly dead leaf, stem, clover, and other non-grass species. Each fraction was fed to a guinea pig. Moderate facial-eczema liver damage was produced by the green material; no visual but slight histologically detectable lesions were obtained from the other fraction. The latter, very mild, damage was thought to be due to small quantities of adventitious green material in the sample.

A sample of highly toxic grass similarly hand sorted has been examined by the beaker test. The green fraction appeared to be almost pure perennial ryegrass, with traces of cocksfoot, and was free from clover, pennyroyal, and other non-grass species. The other fraction consisted only of dead stem and leaf. Both fractions gave beaker tests of intensity 3, which was the same value as for the unsorted pasture. This pasture sample was taken from an area which had been highly toxic for at least some days previously and it is possible that the beaker test material of the brown fraction had been formed while this fraction was still in the green condition. However, from the length of time the brown fraction appeared to have been dead this did not seem very likely. In view of the strong association between beaker-test material and toxicity it is proposed to repeat the earlier dissection experiment on a guinea-pig-feeding scale to find whether the brown fraction could contain appreciable quantities of the facial-eczema poison.

With the exception of the irrigation paddocks (I2 and I3) at the Manutuke Research Station, which were mainly H1 short rotation ryegrass, irrigated during the summer, all areas from which samples were collected were perennial ryegrass, white clover pastures. The Makaraka Racecourse samples were cut over a number of different parts of the grounds, including lawns which contained appreciable amounts of paspalum, plantain, and other species.

Observations on the occurrence of facial eczema on the areas from which grass was collected were as follows:

(i) Claudelands Showgrounds, Makaraka Racecourse, 2 Waikato farms, one Gisborne farm. No sheep grazed on, or in, paddocks adjoining collection area during sampling period.

(ii) Ruakura Animal Research Station. Liver damage in lambs in 1956, but none during period of grass collection in 1957.

(iii) Other Waikato areas. No liver damage in lambs in 1957 from three of these areas. Mild liver damage in lambs grazing adjoining, unknown, fields in 4 other areas. From only one of the latter areas, which was also the one with the greatest liver damage in lambs, was the cut pasture found to be toxic to guinea pigs. (D.S., in Fig. 1.)

(iv) Farm A, Gisborne area. Liver damage in 1957 in grazing sheep.

(v) Farm S, Gisborne area. Serious clinical outbreak of facial eczema in sheep commenced on 5 April 1957.

(vi) Manutuke Research Station. Liver damage in lambs was common in 1956. Clinical cases were observed from 19 March 1957 in lambs rotationally grazing fields 1-5 and considerable liver damage was also found.

These observations confirm and extend the earlier conclusions (Perrin 1957) that the guinea pig is sensitive to the liver-damaging substance which causes facial eczema. They also indicate a general correlation between liver damage in grazing sheep and the results obtained by the beaker test.

No information has so far been obtained concerning the relative stabilities in dried pasture of the white material of the beaker test and the facial-eczema poison.

Although all toxic samples examined have been from ryegrass-dominant pastures there is as yet no evidence to indicate whether the beaker-test material and facial-eczema toxicity are associated solely or mainly with ryegrass species.

Until a more precise method is developed, based, perhaps, on the actual chemical estimation of the facial-eczema poison, the chemical procedure discussed above can be used to provide a sufficiently reliable indication of facial-eczema toxicity of pasture to enable field studies to be undertaken with some confidence. However, this test is empirical and, until more information is obtained concerning the occurrence of the white material and the facial-eczema poison, confirmation of the toxicity of all samples tested in this way should subsequently be sought by guinea-pig feeding.

ACKNOWLEDGEMENTS

The author expresses his thanks to Mrs. J. Sandos and Miss G. Hughes for assistance in carrying out many of the analyses, to Mr. E. P. White for providing and identifying most of the pure species tested, to Mr. N. M. Lauriston for solvent preparation and distillation, and to Mr. E. A. Schoenberger for supervision of most of the guinea-pig feeding. Dr. A. H. Carter has provided valuable assistance in critically examining the data and interpretations.

REFERENCES

Perrin, D. D. 1957: *N.Z. J. Sci. Tech. A38:* 669.

White, E. P. 1958: *N.Z. J. Agric. Res. 1:* 433.

A COMPARISON OF THE VITAMIN B_{12} AND COBALT CONTENTS OF LIVERS FROM NORMAL LAMBS, COBALT-DOSED LAMBS, AND OTHERS WITH A RECENT HISTORY OF MILD COBALT DEFICIENCY DISEASE

By E. D. Andrews, L. I. Hart, and B. J. Stephenson,
Department of Agriculture, Animal Research Station, Wallaceville

(Received for publication, 7 November 1958)

Summary

Vitamin B_{12} and cobalt concentrations were determined in the livers of 3 groups of lambs. One group, not given supplementary cobalt, was grazed on cobalt-sufficient pasture. A second group was dosed at the rate of 7 mg Co a lamb a week and grazed on pasture that had remained generally low in cobalt until about 2 months before the end of the trial. The third group was given no supplementary cobalt, grazed the same pasture as the second group and showed evidence of mild cobalt deficiency disease followed by a recovery within the last 2 months of the trial.

The mean vitamin B_{12} value for livers from unsupplemented lambs on cobalt-sufficient pasture was 0.65 μg/g of wet tissue. Cobalt dosing approximately doubled the mean vitamin B_{12} concentration in livers from lambs grazing pasture previously low in cobalt to a level approaching that for unsupplemented lambs on cobalt-sufficient pasture. The mean vitamin B_{12} value for livers from the group with a previous history of cobalt deficiency was 0.31 μg/g. Consideration of all the results suggests that cobalt deficiency in individual lambs is not likely to be associated with liver vitamin B_{12} concentrations ranging upward from about 0.30 μg/g, but concentrations of the order of 0.20 to 0.30 μg/g could be consistent with a recent history of mild cobalt deficiency.

Most (in some cases virtually all) of the cobalt in the livers of lambs on cobalt-sufficient pasture which had not received supplementary cobalt was in the form of vitamin B_{12}. By contrast most of the cobalt in the livers of cobalt-dosed lambs tended to be in some form other than vitamin B_{12}. There was some indication that cobalt deficiency had resulted in a greater reduction of vitamin B_{12} cobalt than of non-vitamin B_{12} cobalt.

Introduction

Since 1950, various experiments connected with cobalt-deficiency disease in lambs have been carried out at the Winton Experimental Farm, Southland. These include a long-term investigation still current to determine whether cultivation and cropping will affect the cobalt status of the soil and, following re-grassing, the subsequent thrift of lambs. In another trial during the 1955-56 season an attempt was made to test the effectiveness of large doses of cobalt given at relatively long intervals. While it is not the purpose of this paper to describe these two trials in detail, information derived from them enabled us to make comparisons of the vitamin B_{12} and cobalt concentrations in livers from a group of normal lambs not given supplementary cobalt, a group of cobalt-dosed lambs, and a third group, which, it was considered, had shown a recent history of mild cobalt deficiency disease. These results

form part of a brief report published elsewhere (Andrews, Hart, and Stephenson, 1958) and are discussed in detail in this paper.

EXPERIMENTAL

Trial Paddocks

Two paddocks, No. 11 ($1\frac{1}{3}$ acres) and No. 7 (5 acres) were used.

Paddock 11 produced cobalt-deficient lambs in 1952 under light-grazing conditions but not in 1953 when pastures were kept short by heavy grazing (Andrews, Stephenson, Anderson, and Faithful 1958). In the following season paddock 11 was ploughed, and, in April 1954, resown to a permanent-pasture mixture containing short-rotation and perennial ryegrasses, cocksfoot, timothy, and red and white clovers. The cobalt contents of pasture samples taken at approximately monthly intervals between mid September 1954, and the end of March 1955 ranged from 0.17 to 0.24 p.p.m. and were thus well above deficiency levels. Lambs stocked at a rate sufficient to allow ample length of pasture grew satisfactorily and in a controlled trial during May and June 1955 showed no response to cobalt dosing.

Paddock 7 had been in permanent pasture (perennial ryegrass, dogstail, and white clover) since 1948. Between 1951 and 1955 inclusive, pastures had in general shown a low cobalt content and in each of the 5 years had produced cobalt-deficient lambs.

Hence at the start of the 1955-56 season's experiments (with which the present paper is concerned) past histories of the two paddocks suggested that cobalt-deficiency disease would probably not occur in paddock 11 but would very likely do so in paddock 7.

During the summer and autumn period of the present trials white clover was dominant in paddock 11. Pasture in paddock 7 was judged to be of more inferior quality and contained substantially less clover.

Pasture samples for cobalt analyses were taken from each of the trial paddocks in October, November, and December 1955, and in January, February, April, and May 1956.

Experimental Lambs

Romney × Southdown lambs were used and were assigned to their respective paddocks at birth, that is, from about September 1955. Initially, paddock 11 carried 12 lambs and paddock 7 carried 47 lambs. All lambs were weaned on 7 December 1955.

For the purposes of the present paper lambs in paddock 11 are hereinafter designated as Group A. Except for routine shepherding, which for all lambs included crutching and a dose of phenothiazine on 2 May 1956, lambs in Group A received no special treatment. They were first weighed on 8 December 1955 and weighings were continued at fortnightly intervals up to and including 16 May 1956.

Lambs in paddock 7 were first weighed on 7 December 1956. At that time they were divided into 4 lots, as evenly as possible, on a body-weight basis, and given the first of the following treatments.

Lot (1) Cobalt, 280 mg a lamb every 4 weeks (12 lambs)
(2) Cobalt, 28 mg a lamb every 2 weeks (11 lambs)
(3) Cobalt, 7 mg a lamb a week (12 lambs)
(4) Control (12 lambs)

Cobalt was given as a one-ounce dose containing the appropriate amount of cobalt sulphate in aqueous solution. During February 1956 it was noted that a number of lambs, including some in each lot receiving cobalt, had developed clinical ryegrass staggers, but within the following month all obvious signs of this disease had disappeared. It is of incidental interest that in contradistinction to the beneficial effect of cobalt in preventing phalaris staggers (Lee and Kuchel 1953) cobalt dosing failed to prevent ryegrass staggers. Except for one weighing missed 13 weeks after the trial started, all lambs in paddock 7 were weighed at weekly intervals up to and including 22 May 1956. During the trial period the three lots of lambs receiving cobalt showed a broadly similar pattern of mean weight changes. The weight changes made by all cobalt-dosed lambs were used to calculate the significances of mean differences between cobalt-treated and untreated control lambs. Otherwise, those lots of lambs receiving 280 mg and 28 mg cobalt are not considered further here. For the purposes of the present paper those lambs in paddock 7 which received 7 mg cobalt a week are designated as Group B, while the control lot are designated as Group C.

Lambs from both paddocks were killed at the freezing works on 23 May 1956, and the livers from Groups A, B, and C were taken for vitamin B_{12} and cobalt determinations.

Pasture and Liver Examinations

Cobalt concentrations in pasture and liver samples were determined by a Nitroso-R-salt spectrophotometric method (McNaught 1948b). Vitamin B_{12} was extracted from homogenised liver by steaming with cyanide (Analytical Methods Committee of the Society for Analytical Chemistry, 1956) and determined in the extracts by microbiological assay using *Lactobacillus leichmannii* ATCC 7830 (Krieger 1954).

In conformity with usual practices, cobalt concentrations are expressed here as p.p.m. on a dry-weight (d.w.) basis and vitamin B_{12} concentrations as μg/g on a wet-weight (w.w.) basis. Vitamin B_{12} cobalt/total cobalt ratios for livers were estimated from corresponding vitamin B_{12} values converted to a dry-weight basis, and the cobalt content of the vitamin (4.3%) as determined by its formula (Hodgkin *et al.* 1955).

Results

Cobalt Contents of Pastures and Lamb Weight Changes

In a previous paper (Andrews, Stephenson, Anderson, and Faithful 1958) the "minimal level" of cobalt in pasture was defined as the lowest average concentration which would continuously satisfy the cobalt requirement of grazing sheep. It was considered that 0.11 p.p.m. approximated the minimal level of pasture-associated cobalt required by lambs.

Results of analyses of pasture samples from paddocks 11 and 7 are shown in Fig. 1. Also shown are mean weight changes of lambs in paddock 11 (Group A), and of Group B (7 mg Co/lamb/week) and Group C (control) in paddock 7.

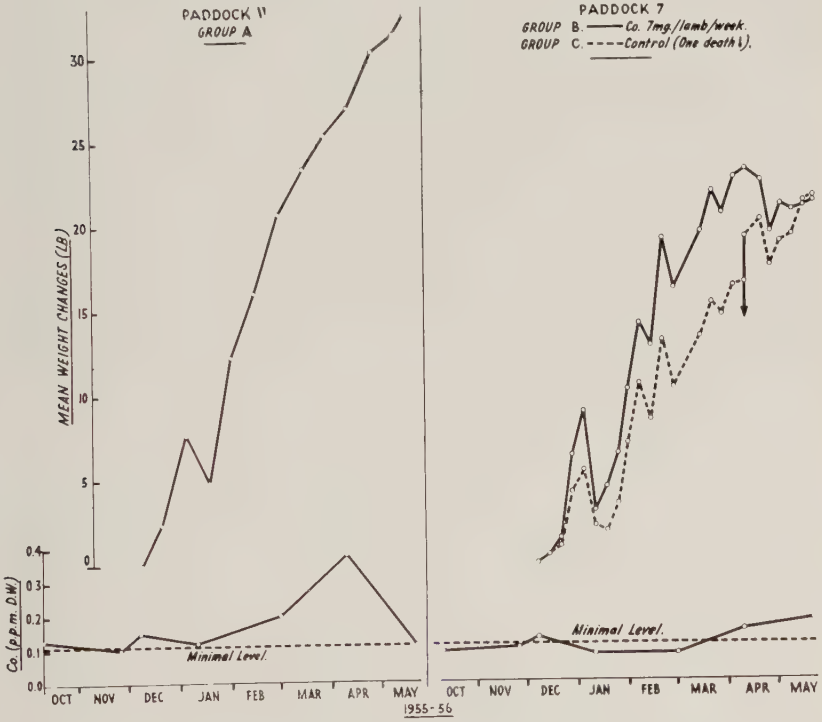


Fig. 1.—Cobalt content of pasture samples and weight changes of experimental groups of lambs.

It appears that the pasture cobalt available to lambs in Group A was in general adequate throughout the experimental period. Except for a set-back of unknown cause in January, all lambs in Group A grew normally. This, together with the previous history of the paddock and analytical results for pasture samples, indicates that cobalt deficiency was not a factor limiting the growth of lambs in Group A.

From the results of analyses of pasture samples from paddock 7, it appears that pastures in this paddock on an average contained insufficient cobalt for lambs from October 1955 to March 1956. Results for April and May 1956, however, suggest that adequate cobalt was available from pasture during the last 2 months of the trial. However, as exemplified in Fig. 1, growth rates of both groups of lambs in paddock 7 were erratic throughout the trial and on 16 May the mean gain for Group B lambs was 11.5 lb less than the corresponding mean gain for Group A lambs in paddock 11. Since, as far as is known, 7 mg weekly doses of cobalt will effectively control cobalt-deficiency disease

in sheep (Lee 1950) it seems clear that, irrespective of treatments, the growth of lambs generally in paddock 7 was limited by some factor or factors other than cobalt-deficiency disease. Comparisons of mean gains for Groups B and C showed that on 15 March there was a 6.6 lb difference in favour of the former. Although this difference did not attain statistical significance ($0.05 < P < 0.1$) the difference (5.7 lb) at this time between the mean for all cobalt-dosed lambs and that for the untreated group was significant ($P < 0.05$). In mid April, one untreated lamb died after losing weight in a manner suggestive of cobalt deficiency. When the trial was terminated on 22 May the mean gain for all cobalt-dosed lambs was 23.1 lb and that for survivors in Group C was 21.7 lb. The small difference (1.4 lb) was not significant. From the previous history of the paddock, the evidence afforded by pasture analyses, and comparisons of weight gains of cobalt-dosed and untreated lambs, we conclude that mild cobalt deficiency had developed among the latter by March. Thereafter, an apparent recovery took place during the last 2 months of the trial. These results confirm previous reports (Dixon 1936; Wünsch 1937) suggesting that, in Southland, lambs may recover from cobalt deficiency during autumn in the same areas in which earlier they developed the disease.

Vitamin B_{12} and Cobalt in Livers

Weight gains of individual lambs surviving from the start of weighing until 16 May, vitamin B_{12} and cobalt concentrations, and B_{12}Co/total Co ratios calculated from the corresponding vitamin B_{12} and cobalt figures, are set out in Table 1. For ease of comparison, vitamin B_{12} values for each group are arranged in descending order of magnitude.

Vitamin B_{12}. For group A, vitamin B_{12} concentrations range from 0.39μg/g to 1.11 μg/g with a mean of 0.65 μg/g. Ranges and the mean for cobalt-dosed lambs (Group B) are of a similar order.

The relatively high figure for lamb 4214 in Group C lies well outside the range of values found for the rest of the group. When weighings commenced, this lamb weighed less than others in the group, but subsequently, as shown in Table 1, made the greatest gain. Although vitamin B_{12} ranges for Groups B and C overlap, the mean for the former group is nearly twice that found for the latter and the difference between the two means is statistically very highly significant ($P < 0.001$).

Cobalt. In terms of the criteria used at Wallaceville for assisting in the diagnosis of cobalt deficiency disease (McNaught 1948a; Andrews, Stephenson, Anderson, and Faithful 1958) the mean cobalt concentration for livers from Group A lambs is within the normal range. Nevertheless, the cobalt content of livers from 3 lambs (Nos. 4261, 4269, 4271) in this group would be classified as borderline. The weight gains of these 3 lambs were in each case less than the mean for the group, but there is no positive evidence from the results that they had been affected by cobalt-deficiency disease.

The very high cobalt figures for lambs 4210 and 4229 in Group B are clearly not typical of results found for the rest of the group. The

TABLE 1. VITAMIN B_{12} AND COBALT CONCENTRATIONS IN LIVERS

Group	Treatment	Cobalt Status	Lamb No.	Wt. Gain (lb)	Liver Determinations		
					Vitamin B_{12} (μg/g w.w.)	Cobalt (p.p.m. d.w.)	B_{12} Co/ Total Co
A	None	Sufficient	4259	26	1.11	0.15	1.0
			4262	44	0.93	0.15	1.0
			4253	41	0.84	0.16	0.7
			4268	35	0.82	0.12	0.9
			4251	35	0.75	0.12	0.8
			4258	19	0.63	0.10	0.8
			4256	31	0.56	0.10	0.8
			4257	51	0.55	0.11	0.7
			4261	28	0.44	0.09	0.7
			4272	30	0.43	0.10	0.7
			4269	30	0.39	0.08	0.7
			4271	21	0.39	0.08	0.7
			Means	33	0.65	0.11	0.8
B	7 mg Co weekly	Sufficient	4218	30	0.96	0.31	0.5
			4215	25	0.82	0.35	0.4
			4209	18	0.69	0.27	0.4
			4208	23	0.64	0.33	0.3
			4210	17	0.56	6.41*	<0.02*
			4204	22	0.54	0.32	0.3
			4236	26	0.53	0.27	0.3
			4229	16	0.52	3.94*	<0.02*
			4220	28	0.50	0.34	0.2
			4237	14	0.47	0.34	0.2
			4241	18	0.36	0.33	0.2
			4202	17	0.32	0.60	0.1
			Means	21	0.58	0.35	0.3
C	None	Recovered from mild deficiency	4214	31	0.60	0.12	0.8
			4225	19	0.37	0.07	0.7
			4242	30	0.34	0.07	0.7
			4246	30	0.31	0.08	0.6
			4243	23	0.31	0.08	0.6
			4230	23	0.29	0.05	0.9
			4231	23	0.28	0.07	0.6
			4213	7	0.27	0.08	0.5
			4240	17	0.26	0.06	0.6
			4234	20	0.22	0.08	0.4
			4223	12	0.19	0.07	0.4
			Means	21	0.31	0.08	0.6

* Atypical values, not included in calculations of means.

reason for these abnormally high figures is not known. Lamb 4210 was known to have been one of two in the group definitely affected by rye-grass staggers. The other was lamb 4202 for which the cobalt figure is

also relatively high. Although the weight gains of lamb 4210 and lamb 4229 are in each case less than the mean for the group, one other lamb (No. 4237) made less gain. If results for the two former lambs are neglected, cobalt concentrations for Group B lambs range from 0.27 to 0.60 p.p.m. and the mean (0.35 p.p.m.) is about 3 times higher than that found for Group A. Of the cobalt figures for Group C, one result (Lamb 4214) would be classified as normal, 8 as borderline (0.07–0.09 p.p.m.) and two as low (<0.07 p.p.m.).

B_{12} Co/total Co ratios. For Group A, values range from 0.7 to 1.0 with a mean of 0.8. For Group B, corresponding figures are obviously very much lower. Results for Group C range from 0.4 to 0.8 with a mean of 0.6.

Discussion

Vitamin B_{12} Concentrations in Livers of Lambs Not Affected by Cobalt Deficiency

Although the cobalt content of 3 livers from lambs in Group A would be classified as borderline, we consider that the weight of other evidence indicates that the cobalt intake of all lambs was probably adequate for normal growth. Factors other than cobalt deficiency affected the growth rates of at least some lambs in Group B. Whether the relatively inferior pasture in paddock 7 (as judged by its clover content) was responsible for this, or whether ryegrass staggers played some part in reducing rates of weight gain, is not known. But in the light of existing knowledge, lambs in Group B must also be regarded as normal insofar as cobalt dosing is presumed to have met their vitamin B_{12} requirements. In the present experiment, vitamin B_{12} concentrations in the livers of lambs dosed with cobalt were of a similar order to those found for normal lambs receiving adequate cobalt from pasture. In general, it appears that cobalt deficiency is not associated with liver vitamin B_{12} concentrations ranging upwards from about 0.30 μg/g.

Effect of Cobalt Dosing on Vitamin B_{12} Concentration

Since lambs in Group C acted as controls to those in Group B, it is apparent that cobalt dosing resulted in an approximately twofold increase in the mean vitamin B_{12} concentration in liver to a level comparable to that found for untreated normal lambs. American (Hoekstra *et al.* 1952; Kercher and Smith 1956) and Russian (Koval'skii and Raetskaya 1955) workers also report increased liver storage of vitamin B_{12} in response to cobalt dosing.

Vitamin B_{12} Concentrations in the Livers of Recently Cobalt-deficient Lambs

Since lambs differ in susceptibility to cobalt deficiency and, on a group basis, the degree of previous deficiency among Group C lambs was mild, it is probable that the growth rates of some lambs in this group remained unaffected. Individual weight changes either between December and March (not shown in Table 1) or between December and mid May (Table 1) do not, however, provide a sufficiently sensitive means of distinguishing with certainty between those animals

whose growth rates had been impaired by the disease and those whose growth rates had not. It is not, of course, known to what extent vitamin B_{12} concentrations in the livers of lambs in Group C increased during the recovery stage. But in terms of the generalisation made above it appears probable that the growth rates of those lambs with liver vitamin B_{12} concentrations exceeding 0.30 μg/g were not appreciably, if at all, reduced by cobalt deficiency. Conversely, previously affected lambs were probably among those with liver vitamin B_{12} concentrations lower than 0.30 μg/g. The fact that the mean weight gain of those 5 lambs in Group C whose liver vitamin B_{12} concentrations exceeded 0.30 μg/g was nearly 10 lb greater than the mean gain for the 6 lambs whose livers contained less than 0.30 μg/g supports this view. The lowest vitamin B_{12} figure found was 0.19 μg/g. To generalise, results suggest that liver vitamin B_{12} concentrations of the order of 0.20 to 0.30 μg/g can be consistent with a recent history of mild cobalt-deficiency disease in individual lambs.

Comparisons of B_{12} Co/Total Co Ratios

From results for Group A it appears that most, and in some cases virtually all, of the cobalt in the livers of normal grazing lambs not receiving supplementary cobalt was in the form of vitamin B_{12}.

If mean vitamin B_{12} and cobalt figures for Group A are compared with those for Group C it is evident that, while cobalt dosing raised the mean vitamin B_{12} concentration about twofold, the mean cobalt concentration was increased about fourfold. This is reflected in the B_{12} Co/total Co ratios for Group B which show that in cobalt-dosed sheep most of the liver cobalt tended to be in some form other than vitamin B_{12}. This seems best explained as due to inability of the rumen organisms to convert more than a fraction of inorganic cobalt given as a 7 mg dose to vitamin B_{12}.

A comparison of B_{12} Co/total Co ratios for Groups A and C suggests that cobalt deficiency may tend to result in a greater reduction of vitamin B_{12} cobalt in the liver than of non-vitamin-B_{12} cobalt. This observation has been extended in later work briefly described elsewhere (Andrews, Hart, and Stephenson 1958).

ACKNOWLEDGEMENTS

We are indebted to Staff of the Animal Industry Division, Department of Agriculture, Invercargill, for weighing and dosing of experimental lambs and for assistance with collection of liver samples. The Winton Experimental Farm Committee kindly provided facilities for the work. We are indebted also to Mr. T. G. Robertson, biometrician, Department of Agriculture, Wellington, for statistical examination of lamb weight gains. Fig. 1 was constructed by Mr. A. L. Bryant, Wallaceville.

REFERENCES

Analytical Methods Committee of the Society for Analytical Chemistry. Report of the Vitamin B_{12} Panel. 1956: *Analyst 81:* 132-6.

Andrews, E. D.; Stephenson, B. J.; Anderson, J. P.; Faithful, W. C. 1958: *N.Z. J. agric. Res. 1:* 125-39.

Andrews, E. D.; Hart, L. I.; Stephenson, B. J. 1958: *Nature, Lond. 182:* 869.
Dixon, J. K. 1936: *N.Z. J. Sci. Tech. 17:* 600-19.
Hodgkin, Dorothy C.; Pickworth, Jenny; Robertson, J. H.; Trueblood, K. N.; Prosen, R. J.; White, J. G.; Bonnett, R.; Cannon, J. R.; Johnson, A. W.; Sutherland, I.; Todd, A. R.; Lester Smith, E. 1955: *Nature, Lond., 176:* 325-30.
Hoekstra, W. J.; Pope, A. L.; Phillips, P. H. 1952: *J. Nutr. 48:* 421-30.
Kercher, C. J.; Smith, S. E. 1956: *J. anim. Sci. 15:* 550-8.
Koval'skii, V. V.; Raetskaya, Yu. I. 1955: *Doklady Akad. Nauk. S.S.S.R. 100:* 1131-4. (Seen only in *Chem. Abstr.* 49: 10457 e.)
Krieger, C. H. 1954: *J. Ass. off. agric. Chem. 37:* 781-92.
Lee, H. J. 1950: *Aust. vet. J. 26:* 152-9.
Lee, H. J.; Kuchel, R. E. 1953: *Aust. J. agric. Res. 4:* 88-99.
McNaught, K. J. 1948a: *N.Z. J. Sci. Tech. A30:* 26-43.
————1948b: *Ibid. A30:* 109-15.
Wünsch, D. S. 1937: *Chem. Ind. 15:* 855-9.

THE EFFECTS OF SEASON AND WEATHER ON THE GROWTH RATE OF A RYEGRASS AND CLOVER PASTURE

By R. W. Brougham, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 14 October 1958*)

Summary

An experiment is described in which the effects of seasonal climate and of temporary (weekly) weather variations on the growth rate of a pasture of short-rotation ryegrass and white clover were determined.

The technique of measurement included replication in time as well as space.

Statistical analyses showed significant positive correlations between seasonal trends in growth rate and light and temperature.

Positive correlations were also obtained for the weekly fluctuations in growth rate attributable to weather factors. The pasture was irrigated during the summer and early autumn so that water was at all times adequate for growth.

The average daily growth rate of the pasture ranged from 10 lb of dry matter (d.m.) per acre in the winter to 120 lb per acre in early summer. The weekly fluctuations in growth rate attributable to weather factors were as large as ± 50% of the weekly growth rate.

The potential annual yield of d.m. obtainable from this pasture type growing in this locality, was estimated as 22,000 lb per acre.

Introduction

In a previous experiment (Brougham 1955), the effects of variations in weather on the rate of growth of a pasture of short-rotation ryegrass (*Lolium perenne* × *L. multiflorum*) and white clover (*Trifolium repens*) were determined, by using a field and mathematical technique to separate growth into components attributable to short term changes in weather ('*w*' parameters) and the estimated growth of the pasture under a smoothly changing climate ('*g*' parameters) (Glenday 1955). The previous experiment was a test of the suitability of the technique for field growth studies and was of nine weeks duration. The present study makes use of the technique to determine the influence of climatic factors on the growth rate of this pasture type throughout the year.

The experiment was carried out to derive a set of annual growth curves of total herbage, ryegrass, and white clover, with the influence of weather fluctuations removed. This would enable the potential annual yield of d.m. to be determined for this pasture growing in this locality. Such quantitative information, for pasture species, growing separately and in combination, in different environments, is fundamental to an understanding of the growth of pasture. It is also an essential complement to the results obtained from studies under controlled environmental conditions.

Experimental Methods

The experiment was carried out at the Palmerston North station of the Grasslands Division, Department of Scientific and Industrial Research, on Manawatu silt loam. At the beginning of the experiment, on 12 May 1955, the pasture was in its fourth year from sowing.

The experimental technique has been previously described (Brougham 1955: Glenday 1955). However, there were some modifications made necessary by the extended duration of the present experiment. The interval between time replicates was increased to two weeks, and twenty-nine separate time replicates were started. The first series was closed on 12 May 1955, and the last on 7 June 1956. Each of the time replicates was also replicated in space three times and each was large enough to contain the fourteen sub-plots, 7 ft × 4 ft, necessary for fourteen-week growth curves. Measurements of herbage yield began on 16 June 1955 and the experiment was concluded on 21 June 1956.

Preliminary treatment of the pasture was similar to that previously described (Brougham 1955). However, the interval between preliminary cuts was extended to two weeks.

Herbage yields were determined by cutting a 2-ft-wide strip through each plot and weighing the material collected. The herbage was cut to 1 in., was mixed, and samples taken for d.m. determinations and herbage analyses.

The experimental area was irrigated with an overhead irrigation plant during periods of low rainfall. This was carried out to minimise the possibility of any of the growth fluctuations being attributable to limited water supply (see Fig. 2).

The weather records presented, with the exception of the radiation measurements, were obtained from the official climatological station situated approximately 50 yd from the experimental site. The instruments used in this station are standard equipment for New Zealand (New Zealand Meteorological Official Circ. No. 18, June 1943). The solar radiation measurements were obtained from an Eppley pyrheliometer located on top of a nearby building. Unfortunately, the recording equipment of this instrument was changed during the course of the experiment and, for this reason, weekly averages of the daily total solar radiation cannot be expressed in absolute units. However, an approximate scale is given and has been calculated on the basis that in mid-summer at Palmerston North the total solar radiation, on a cloudless day, is approximately 750 cal/cm^2 of horizontal surface.

Results

Table 1 shows yields of total herbage, ryegrass, and clover, for the twenty-nine growth curves. Although fourteen successive weekly yield determinations were made for each of the time replicates, only the data for the first six-weeks growth of all curves were included in the mathematical analyses. The data obtained at later stages of growth were considered too variable to be included in the analyses. However, the additional information obtained allowed estimates to be made of yield

ceilings, and the age of the pasture when these were reached to be determined. The ceilings are shown in the last column in Table 1 and the age of the pasture in weeks is shown in parenthesis. The mathematical treatment of the results is described by Glenday (1959).

The data presented in Table 1 were separated into growth for a smoothly changing climate and weather effects. Fig. 1 shows such separations of total herbage of the first six weeks growth of each of the time replicates between the dates 12 May 1955 and 26 April 1956. The incomplete data for the last three time replicates were not included in the analysis. The twenty-six separate sets of 'w' parameters obtained from the above analyses were combined into one set (Glenday 1959) and are presented as week by week deviations about a mean of zero. Seasonal trends have also been removed.

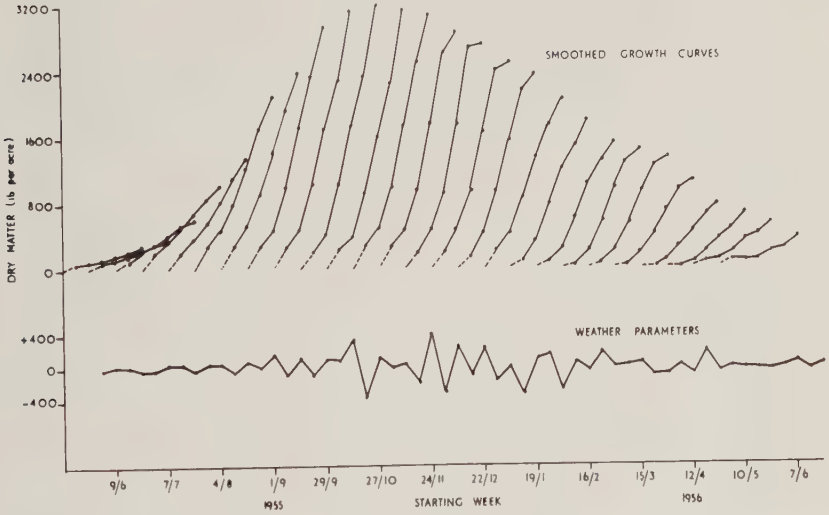


Fig. 1.—Total herbage yields separated into growth curves for a smoothly changing climate and 'w' parameters showing growth attributable to weekly fluctuations in weather.

For the first 4 sets of data the standard errors of estimate of points on the calculated growth curves averaged ± 15.0, rising to ± 95.4 for the middle 4 sets and dropping to ± 33.7 for the final sets. The standard errors of estimate of the 'w' parameters were 22% higher.

Fig. 2 shows the 'w' parameters plotted against the weekly averages of the maximum and minimum daily temperatures, daily total solar radiation, and the amount of rainfall plus irrigation per week for the period 13 October 1955 to 26 April 1956.

Fig. 3 shows the ryegrass and clover data presented in Table 1, each separated into growth for a smoothly changing climate and weather effects. The separation for the clover data before the closing date beginning 13 October 1955 is not presented because the clover com-

TABLE 1. MEAN YIELDS OF TOTAL HERBAGE FOR EACH REPLICATE IN TIME
(Yields are successive measurements of total herbage given in lb d.m. per acre)

Week of Closure	Weeks of Growth						Yield Ceilings and Age of Pasture in Weeks
	1	2	3	4	5	6	
1955							
12.5	—	—	—	—	258	311	1150 (14)
26.5	—	—	131	166	151	223	1900 (14)
9.6	109	114	105	116	241	241	2350 (14)
23.6	41	64	92	111	162	262	3000 (14)
7.7	74	94	158	290	393	538	4650 (14)
21.7	96	203	256	442	636	1016	5200 (14)
4.8	85	177	372	731	1180	1677	6400 (14)
18.8	89	343	587	1137	1608	1953	6900 (14)
1.9	183	299	523	1059	1713	2860	8150 (14)
15.9	193	456	1141	2282	2680	3231	7900 (14)
29.9	116	414	838	1615	2227	3386	7650 (13)
13.10	183	448	1045	1572	1989	3204	5550 (11)
27.10	163	466	867	1836	2425	3093	5550 (10)
10.11	166	474	801	1676	2739	3185	4500 (9)
24.11	167	584	1016	2160	3097	3286	3550 (8)
8.12	281	803	1488	2406	3151	2939	3450 (7)
22.12	224	647	807	1561	2179	2353	2650 (6)
1956							
5.1	148	390	1072	1364	1603	1781	2150 (6)
19.1	256	387	751	1054	1499	1825	1820 (6)
2.2	174	303	769	1290	1539	1932	1940 (6)
16.2	119	358	884	1355	1713	1753	1760 (6)
1.3	139	445	811	1177	1459	1287	1540 (8)
15.3	57	139	291	542	824	911	1020 (7)
29.3	73	98	407	594	841	1038	1140 (9)
12.4	78	262	481	606	758	806	1160 (*)
26.4	46	130	222	371	376	578	1020 (*)
10.5	102	67	139	189	259	345	*
24.5	90	105	123	239	—	- -	*
7.6	68	44	—	—	—		*

* Not reached.

TABLE 1. (Continued) MEAN YIELDS OF RYEGRASS FOR EACH REPLICATE IN TIME
(Yields are successive measurements given in lb d.m. per acre)

Week of Closure	Weeks of Growth						Yield Ceilings and Age of Pasture in Weeks
	1	2	3	4	5	6	
1955							
12.5	—	—	—	—	198	275	1100 (14)
26.5	—	—	117	152	138	206	1800 (14)
9.6	77	93	88	102	218	207	2100 (14)
23.6	37	57	86	101	150	241	2800 (14)
7.7	61	87	144	266	346	488	4350 (14)
21.7	87	181	243	400	587	944	4550 (14)
4.8	79	151	322	653	971	1495	6300 (14)
18.8	82	316	497	1014	1371	1725	6450 (14)
1.9	164	264	474	939	1541	2533	7700 (14)
15.9	166	393	1010	1913	2370	2953	7250 (14)

TABLE 1. (Continued) MEAN YIELDS OF RYEGRASS

29.9	109	389	757	1499	2030	3087	7100 (13)
13.10	167	385	858	1199	1528	2565	4650 (11)
27.10	151	426	736	1706	2203	2755	4550 (10)
10.11	123	331	629	1133	1899	2216	3050 (9)
24.11	145	487	760	1615	2557	2723	2700 (6)
8.12	207	544	1009	1382	2203	1921	2200 (5)
22.12	197	491	619	935	1280	1080	1550 (7)
1956							
5.1	115	241	478	625	897	525	900 (5)
19.1	147	118	211	152	233	250	250 (6)
2.2	81	90	206	261	213	301	300 (6)
16.2	41	80	98	260	416	429	420 (6)
1.3	79	143	231	260	512	204	720 (8)
15.3	28	56	47	202	131	226	900 (8)
29.3	53	39	192	155	345	568	900 (11)
12.4	32	74	145	249	272	385	780 (*)
26.4	26	68	116	146	246	365	860 (*)
10.5	65	37	73	111	120	225	*
24.5	62	76	80	180	—	—	*
7.6	59	30	—	—	—	—	*

TABLE 1. (Continued) MEAN YIELDS OF CLOVER FOR EACH REPLICATE IN TIME
(Yields are successive measurements given in lb d.m. per acre)

Week of Closure	Weeks of Growth						Yield Ceilings and Age of Pasture in Weeks
	1	2	3	4	5	6	
1955							
12.5	—	—	—	—	53	32	50 (5)
26.5	—	—	9	10	7	10	70 (14)
9.6	28	18	13	11	20	21	240 (14)
23.6	4	5	6	8	10	19	200 (14)
7.7	12	7	12	20	46	49	200 (14)
21.7	7	20	12	40	49	68	460 (14)
4.8	5	23	47	66	209	182	520 (11)
18.8	5	23	73	118	237	216	540 (10)
1.9	19	35	49	119	172	318	600 (11)
15.9	26	62	131	347	301	278	1040 (13)
29.9	6	25	69	111	189	219	1080 (12)
13.10	15	61	181	368	442	616	1660 (13)
27.10	12	19	131	123	222	338	2080 (14)
10.11	43	141	173	499	832	969	2000 (14)
24.11	22	89	238	528	503	552	2280 (14)
8.12	70	253	475	988	908	1000	2720 (14)
22.12	25	149	158	549	858	1187	1940 (14)
1956							
5.1	33	149	575	718	639	1231	1740 (10)
19.1	95	255	477	888	1093	1286	1780 (8)
2.2	89	196	531	960	1240	1550	1700 (8)
16.2	72	248	727	1030	1213	1262	1260 (6)
1.3	51	261	572	832	941	1045	1040 (6)
15.3	30	81	237	302	690	655	760 (7)
29.3	18	51	202	416	483	453	560 (8)
12.4	48	182	333	315	466	408	480 (7)
26.4	19	54	105	71	128	207	220 (4)
10.5	34	30	64	71	134	110	140 (5)
24.5	27	27	39	49	—	—	*
7.6	8	11	—	—	—	—	*

* Not reached.

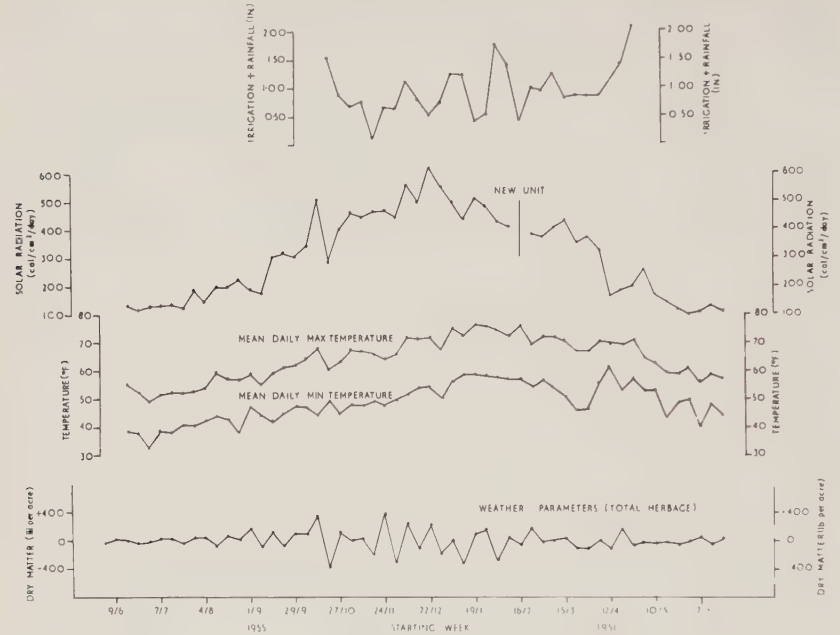


Fig. 2.—Total herbage '*w*' parameters plotted against the various climatological factors.

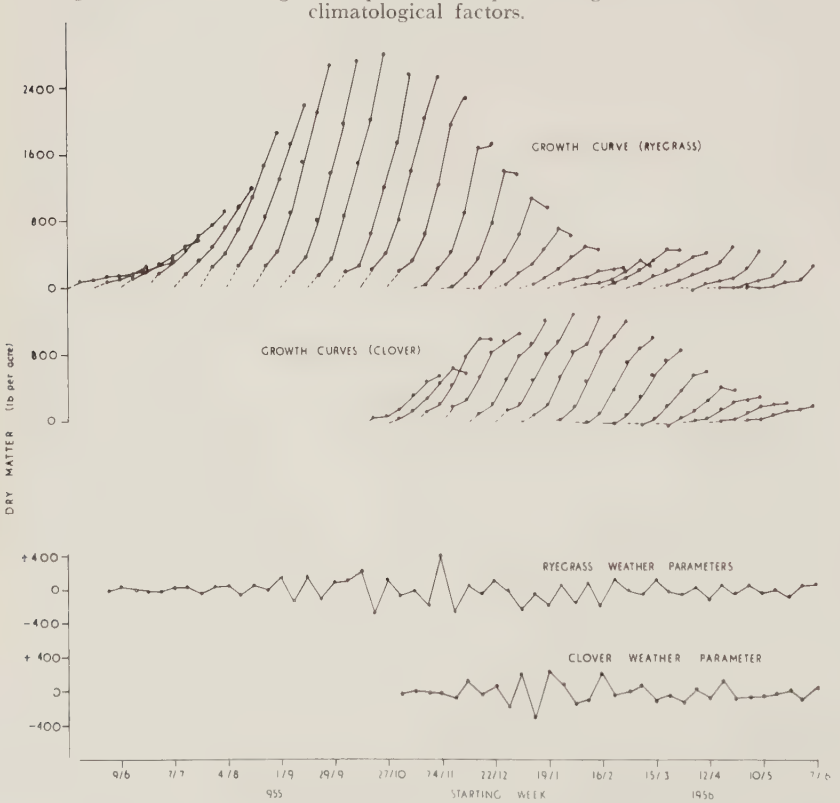


Fig. 3.—Ryegrass and clover yields separated into growth curves for a smoothly changing climate and '*w*' parameters showing growth attributable to weekly fluctuations in weather.

ponent, as a percentage of the total herbage yield, was small. The separate 'w' parameters for ryegrass and clover are presented as week by week deviations about a mean of zero.

Calculations of correlation and regression coefficients relating 'w' parameters to climatological factors were made. The data included in the correlation and regression analyses between the total herbage 'w' parameters and weather factors were for the period 16 June 1955 to 21 June 1956. For ryegrass, it included data for the period 16 June 1955 to 29 December 1955, and for clover, from 5 January 1956 to 29 March 1956. These divisions were determined with reference to the growth curves presented in Fig. 2, and were the periods when each species was dominant. Seasonal trends were removed from the climatological data before calculations of correlations and regressions, by plotting deviations from eleven-point smoothings of each factor. The correlation and regression coefficients are presented in Table 2.

Multiple regression analyses were also performed but these gave no significant improvement over simple regression analyses and are not presented.

The smoothed growth curves presented in Figs. 1 and 3 enable annual curves of total herbage, ryegrass, and clover growth rate to be constructed for pasture at any stage of growth. The data presented in Fig. 4 show curves for the increment in growth between weeks three and four of each time replicate. For total herbage and ryegrass, they include all time replicates, but the clover growth curve includes only the increments between 10 November 1955 and the end of the experiment.

The yield data are plotted as daily d.m. increments, and the dates of each increment are taken as the centre of the twelve weeks used in the parameter fitting process (see Glenday 1959).

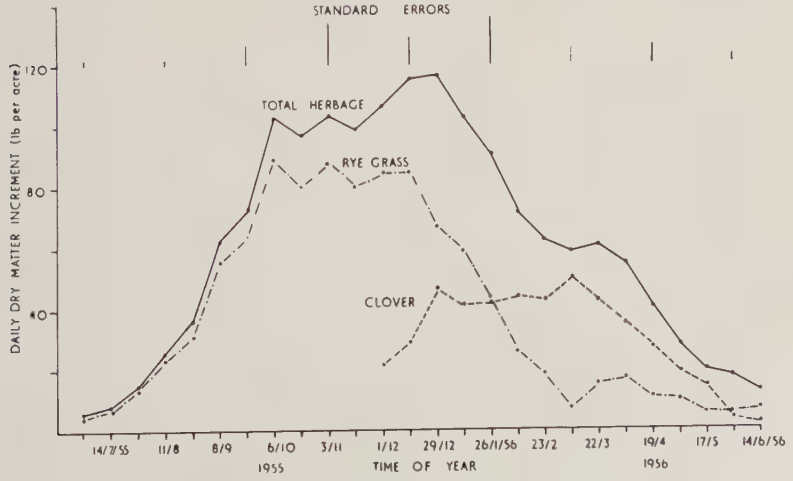


Fig. 4.—Detailed growth curves of total herbage, ryegrass, and clover for the increment in growth between weeks three and four of each time replicate. Standard errors of the total herbage increments are also shown.

TABLE 2. CORRELATION AND REGRESSION COEFFICIENTS RELATING FLUCTUATIONS IN '*w*' PARAMETERS AND CLIMATOLOGICAL FACTORS

Climate Factor	Correlation Coefficient			Regression Coefficient		
	Total Herbage 16.6.55–21.6.56	Ryegrass 16.6.55–29.12.55	Clover 5.1.56–29.3.56	Total Herbage 16.6.55–21.6.56	Ryegrass 16.6.55–29.12.55	Clover 5.1.56–29.3.56
Radiation (cal/cm²)	+ 0.360**	+ 0.470**	+ 0.193	+ 0.93 ± 0.34	+ 1.27 ± 0.46	
Max. temp. (°F)	+ 0.238	+ 0.232	+ 0.622*	—	—	+ 41.1 ± 16.4
Min. temp. (°F)	− 0.092	− 0.099	+ 0.160	—	—	—
Temp. range (°F)	+ 0.308*	+ 0.348*	+ 0.282	+ 16.8 ± 7.3	+ 18.7 ± 9.7	—
Rainfall (in.)	− 0.133	—	—	—	—	—

* $P < 0.05$
** $P < 0.01$

Table 3 shows correlation and regression coefficients relating the seasonal trends in growth rate of total herbage determined as above, to seasonal trends in radiation and air temperature. The data used in these analyses are the successive means of the twelve weeks climatological data used in the fitting, and the yield data are the corresponding yields shown in Fig. 4.

TABLE 3. CORRELATION AND REGRESSION COEFFICIENTS RELATING SEASONAL TRENDS IN GROWTH RATE (total herbage) AND CLIMATOLOGICAL FACTORS

Climate Factor	Correlation Coefficient	Regression Coefficient
Radiation (cal/cm^2)	+ 0.979***	+ 2.05 ± 0.10
Maximum temperature (°F)	+ 0.734***	+ 30.5 ± 6.3
Minimum temperature (°F)	+ 0.611**	+ 29.8 ± 8.8
Temperature range (°F)	+ 0.844***	+125.5 ± 17.8

** $P < 0.01$
*** $P < 0.001$

DISCUSSION

The growth curves of total herbage (Fig. 1) conform to the sigmoid pattern of growth previously described (Brougham 1955). For all seasons, except the winter, the initial phase of growth was of two to three weeks duration. (See Glenday (1959) for an explanation of the effect on the fitted growth curves of possible errors in the final trimming cut of some replicates). This was generally followed by a longer period of growth at a maximum rate. At later stages of growth, the growth rate declined and in some instances yield ceilings were reached by 6 weeks.

The maximum daily rate of growth of total herbage changed markedly with season. This ranged from approximately 10 lb per acre in early July to 120 lb per acre in late December (Fig. 4). A rapid increase is evident in the early spring and by October a growth rate of 100 lb per acre was reached. The decline in growth rate was more gradual over the summer and autumn.

The correlation coefficients relating these seasonal trends in growth rate with those in light and temperature are presented in Table 3. The strongest correlation was between light and growth rate.

Yield ceilings of total herbage, and the time intervals required to reach them (Table 1), also showed marked seasonal trends. The lowest ceilings were recorded in the winter and the highest in the spring. From December until the end of the experiment, there was a decreasing trend in both yield ceilings and the time required to reach these ceilings. The beginning of this trend was associated with the commencement of irrigation and the lowered ceilings are attributed to losses of herbage through tissue decomposition and decay of lodged straw, and the action of saprophytic fungi, slugs, and worms, in the induced moist

environment. In addition, dry matter losses through shedding of ripe seed cannot be overlooked. Similarly, the low yield ceilings obtained during the late autumn and winter are attributed to the action of these destructive agencies (Brougham 1956a).

Changes in the growth rate of ryegrass during the winter and spring months were similar to those of total herbage (Table 1 and Fig. 4). At the onset of summer, however, the ryegrass growth rate decreased rapidly and by late summer and early autumn ryegrass was a negligible component. During this period, the growth rate of the pasture was determined by the clover component.

These changes in growth rate and botanical composition can be explained in terms of species temperature tolerance, inter-species competition for light, seasonal growth patterns of the species, and tissue decomposition. During this experiment, the weekly mean temperature ranged from 40°F (winter) to approximately 70°F (summer). The lowest weekly minimum temperature recorded was 33°F and the highest weekly maximum temperature was 76°F. The results obtained by Mitchell (1956) show that over the temperature range 55°F to 85°F the rate of growth of the individual vegetative tiller of short-rotation ryegrass is relatively constant, although a temperature optimum of 60°F is quoted. On the other hand, white clover has a considerably higher temperature optimum of 75°F, and a smaller favourable range of 65°F to 85°F. At lower temperatures than these (45°F), the growth rate of ryegrass, although reduced, is much higher than that of white clover. From these results it is seen that temperature conditions in this experiment would be more favourable for ryegrass growth over the winter and spring and equally favourable for both ryegrass and clover growth during the summer and autumn. In addition, competition for light among the component species of a pasture would markedly reduce the growth rate of the slower growing component (Blackman 1938; Donald 1956). This is evident from the results presented in Fig. 3 where, in a number of the growth curves, large weekly increases in the growth rate of one component were associated with decreases in the growth rate of the other. It is realised that this may also include slight systematic errors in botanical analyses due to difficulties of field sampling under adverse weather conditions. The marked change in the botanical composition of the pasture that occurred at the beginning of summer can probably be explained by the transition from vegetative to reproductive growth that occurred in ryegrass at this time. The fact that large patches of fungal mycelia were also observed on ryegrass leaves during the irrigation period, suggests that differential tissue decomposition also occurred, with the destructive agencies showing a preference for ryegrass.

The analysis of the relationships between the total herbage 'w' parameters and climatological factors showed a significant positive effect of radiation ($P < 0.01$), and of temperature range ($P < 0.05$). No significant relationship was obtained in the factors analysed. For the ryegrass analysis for the period 16 June 1955 to 29 December 1955, when this was the dominant species of the pasture, similar positive effects were recorded. However, for clover, the relationships between radiation or temperature range, and the ('w') parameters for the period 5 January

1956 to 29 March 1956, were not significant, although a significant positive correlation with maximum temperature (< 0.05) was obtained.

These analyses show that, in this locality during the winter and spring months, weekly fluctuations in the growth rate of ryegrass and consequently total herbage were associated with fluctuations in light and temperature range. However, during the summer and early autumn when clover was the dominant component of the pasture, weekly fluctuations in the growth rate of this species were associated mainly with fluctuations in temperature.

Fluctuations in radiation during the winter and spring were as large as 40% of the mean total daily amount received, and from the evidence produced by Benedict (1941), Blackman and Wilson (1951), Pritchett and Nelson (1951) and Mitchell (1955), fluctuations of this order will markedly affect the growth rate of pasture species. Similarly, weekly fluctuations of up to 10°F in the mean temperature range were recorded. Fluctuations in growth rate associated with these fluctuations in temperature range could therefore be expected. Although correlations between day or night temperature and growth rate were not obtained, the explanation offered by Black (1955) in discussing a correlation between net assimilation rate of subterranean clover and temperature range, that the wider the range of temperature the greater the net assimilation rate, probably follows for the positive correlation obtained here. In discussing these relationships it should be noted that the maximum deviations from the seasonal trends of temperature were of the order of only ± 5°F over the whole experiment.

During the summer and early autumn, the amount of incoming radiation was relatively constant (350–450 cal/cm²/day). For this reason, no significant correlation between weekly fluctuations in growth rate and this factor were obtained. However, although maximum temperatures were within the favourable range 65°F to 85°F for the growth of white clover (Mitchell 1956), night temperatures were much lower and a temperature influence was recorded.

The magnitude of the weekly fluctuations in growth rate attributable to weather factors are of interest. The amount of growth estimated to have been obtained under a smoothly changing climate during the spring and early summer was approximately 700–800 lb d.m. per acre per week (Fig. 1). From Fig. 2, weekly fluctuations in growth of up to ± 400 lb were recorded, i.e. ± 50% of the expected growth. During the winter, fluctuations of a similar relative magnitude were obtained. In a weekly growth rate of 100 lb per acre, fluctuations of ± 50 lb per acre occurred.

The results presented in Table 2 show that, during the winter and spring months, a weekly fluctuation of 1 radiation unit (1 cal/cm²/day), or a change of 1°F in temperature range, were associated with changes in the growth rate of ryegrass of 1.3 and 19 lb of d.m. per acre per week respectively. On the other hand, a 1°F change in maximum temperature during the summer and early autumn was associated with a change in the weekly growth rate of clover of approximately 40 lb d.m. per acre. There was of course a positive correlation between radiation and

temperature. For the complete experimental period, weekly fluctuations in the growth rate of total herbage associated with unit increases in radiation or temperature range were slightly less than the changes in growth rate of ryegrass and clover (Table 2).

The magnitude of these weekly radiation and temperature influences on growth rate, and the fact that positive correlations were obtained, suggest that, under field conditions, the growth rate of pasture responds fairly rapidly to changes in environmental conditions. This rapidity of response under field conditions lends some support to the view held by Mitchell and Coles (1955) that the rate and pattern of tissue formation of pasture species are dependent on current, rather than previous, environmental conditions.

However, when the results presented in Tables 2 and 3 are compared, indirect support is obtained for the conclusions reached by Blackman and Wilson (1954), who showed that a period of adaptation is required for the growth of sunflower when transferred from one light environment to another. A comparison of the regression coefficients shows that the magnitude of changes in the growth rate of pasture attributable to a unit increase in radiation or temperature is much less for the regressions relating short-term fluctuations in weather parameters and climatological data than for those relating the seasonal trends. A possibility is that, although responses to current environmental conditions occurred, the full effect of these changes in environment was not obtained because of the need for a longer adaptive period in plant and soil. It should also be noted that correlations between short-term fluctuations in radiation and temperature are much less than between seasonal trends in those factors, so that short-term effects of one factor are reduced by the frequent lack of concurrent short-term changes in other factors. Current experimental work is aimed at determining the magnitude and lags in pasture growth responses to daily fluctuations in climate (Glenday and Brougham unpublished data).

An estimate of the potential annual yield of a pasture of short-rotation ryegrass and white clover under summer irrigation in this locality is obtainable from the results presented here. The addition of the weekly increments in growth rate between weeks three and four, as shown in Fig. 4, give a total annual yield of approximately 22,000 lb of d.m. per acre. This yield compares with yields of 12–16,000 lb of d.m. per acre obtained from grazed pasture (Sears 1949) using the cage method of measurement. Decreases of this magnitude (25–45%) under grazing conditions can be caused by a number of factors. Two of considerable importance would be losses of herbage through treading damage (Edmond 1958), and incomplete utilisation of light energy through severe defoliations at different times of the year (Brougham 1956b, 1958). Also decreases in growth caused by low available soil moisture during the summer months cannot be overlooked.

A summation of the results obtained from this experiment, particularly those relating growth rate to the various climatological factors, show that, in general, they are in agreement with those obtained by various workers using the growth analysis technique (Briggs, Kidd, and West 1920) for the study of the effect of environmental factors on the

growth of different species. The positive correlations obtained between changes in growth rate and light are in accordance with the results obtained for the growth of sunflower (Blackman, Black, and Kemp 1955), and subterranean clover (Black 1955) when the components of growth of these species were examined. For both species, relative growth rate was shown to be positively correlated with light. Other workers (Gregory 1926; Williams 1939; Watson 1947) have used the growth analysis technique to relate growth to the various environmental factors, but these refer to changes at different stages of vegetative development of the species studied, and are therefore not comparable. For the growth of the ryegrass, the positive correlation obtained between weekly fluctuations in growth rate and temperature range agrees with that obtained for the relative growth rate of subterranean clover, whereas for the white clover the positive correlation between weekly fluctuations in growth rate and maximum temperature is similar to that obtained for the relative growth rate of sunflower. The seasonal trends in the growth rate of the pasture, in this experiment, were also shown to be dependent on the temperature factors, whereas Black (1955) demonstrated that the growth of subterranean clover at Adelaide, Australia, was independent of temperature. The lowest weekly mean temperature recorded at Adelaide was 47.6°F, whereas, in this locality, mean temperatures throughout the year are generally 10°F lower than those at Adelaide. Apparently limiting temperatures to ryegrass and white clover growth occurred at Palmerston North.

Finally, because of the general agreement obtained between these results from the analysis of pasture growing under field conditions, and those of other workers using the growth analysis technique on single plants, the technique used here to determine quantitatively the extent to which seasonal and short term changes in weather influence the growth rate of pasture, has been successful. Considered in this light, the technique is complementary to the growth analysis technique, both of which are relatively easy methods of determining growth responses to changing climatological conditions. It is also a complement to controlled environment studies and will provide basic material for more intensive study under controlled conditions.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the technical assistance of Mr. L. E. Madgwick and Miss D. P. Owen.

REFERENCES

Benedict, H. M. 1941: Growth of Some Range Grasses in Reduced Light. *Bot. Gaz. 102:* 582-9.

Black, J. N. 1955: The Interaction of Light and Temperature in Determining the Growth Rate of Subterranean Clover (*Trifolium subterraneum* L.). *Aust. J. biol. Sci. 8:* 330-43.

Blackman, G. E. 1938: The Interaction of Light Intensity and Nitrogen Supply in the Growth and Metabolism of Grasses and Clover (*Trifolium repens*). 1. The Effects of Light Intensity and Nitrogen Supply on the Clover Content of the Sward. *Ann. Bot. (Lond.) n.s. 2:* 257-80.

Blackman, G. E.; Wilson, G. L. 1951: Physiological and Ecological Studies in the Analysis of Plant Environment. 6. The Constancy for Different Species of a Logarithmic Relationship Between Net Assimilation Rate and Light Intensity and its Ecological Significance. *Ibid. 15:* 373-408.

————1954: Physiological and Ecological Studies in the Analysis of Plant Environment. 9. Adaptive Changes in the Vegetative Growth and Development of *Helianthus annuus* Induced by an Alteration in Light Level. *Ibid. 18:* 71-94.

Blackman, G. E.; Black, J. N.; Kemp, A. W. 1955: Physiological and Ecological Changes in the Analysis of Plant Environment. 10. An Analysis of the Effects of Seasonal Variation in Daylight and Temperature on the Growth of *Helianthus annuus* in the Vegetative Phase. *Ibid. 19:* 527-48.

Briggs, G. E.; Kidd, F.; West, C. 1920: A Quantitative Analysis of Plant Growth. *Ann. appl. Biol. 7:* 103-23.

Brougham, R. W. 1955: A Study in Rate of Pasture Growth. *Aust. J. agric. Res. 6:* 804-12.

———1956a: The Rate of Growth of Short-rotation Ryegrass Pastures During the Late Autumn, Winter, and Early Spring. *N.Z. J. Sci. Tech. A38:* 78-87.

———1956b: Effect of Intensity of Defoliation on Regrowth of Pasture. *Aust. J. agric. Res. 7:* 377-87.

——1958: Interception of Light by the Foliage of Pure and Mixed Stands of Pasture Plants. *Ibid. 9:* 39-52.

Donald, C. M. 1956: Competition Among Pasture Plants. *Proc. 7th int. Grassl. Congr.:* 80-91.

Edmond, D. B. 1958: The Influence of Treading on Pasture. A Preliminary Study. *N.Z. J. agric. Res. 1(3):* 319-28.

Glenday, A. C. 1955: The Mathematical Separation of Plant and Weather Effects in Field Growth Studies. *Aust. J. agric. Res. 6:* 813-22.

————1959: Mathematical Analysis of Growth Curves Replicated in Time. *N.Z. J. agric. Res. 2:* 297-305.

Gregory, F. C. 1926: The Effect of Climatic Conditions on the Growth of Barley. *Ann. Bot. (Lond.) 40:* 1-26.

Mitchell, K. J. 1955: Growth of Pasture Species. 2. Perennial Ryegrass (*Lolium perenne*), Cocksfoot (*Dactylis glomerata*), and Paspalum (*Paspalum dilatatum*). *N.Z. J. Sci. Tech. A37:* 3-26.

——1956: The Influence of Light and Temperature on the Growth of Pasture Species. *Proc. 7th int. Grassl. Congr.* 58-69.

Mitchell, K. J.; Coles, S. T. J. 1955: Effects of Defoliation and Shading on Short-rotation Ryegrass. *N.Z. J. Sci. Tech. A36:* 586-604.

Pritchett, W. L.; Nelson, L. B. 1951: Effect of Light Intensity on the Growth Characteristics of Alfalfa and Bromegrass. *Agron. J. 43:* 172-7.

Sears, P. D. 1949: The Technique of Pasture Measurement. *N.Z. J. Sci. Tech. A33:* 1-29.

Watson, D. J. 1947: Comparative Physiological Studies on the Growth of Field Crops. 1. Variations in Net Assimilation Rate and Leaf Area Between Species and Varieties and Within and Between Years. *Ann. Bot. (Lond.) n.s. 11:* 41-89.

Williams, R. F. 1939: Physiological Ontogeny in Plants and its Relation to Nutrition. 6. Analysis of the Unit Leaf Rate. *Aust. J. exp. biol. med. Sci. 17:* 123-32.

MATHEMATICAL ANALYSIS OF GROWTH CURVES REPLICATED IN TIME

By A. C. Glenday, Applied Mathematics Laboratory, Department of Scientific and Industrial Research, Wellington

(*Received for publication, 14 October 1958*)

Summary

Alternative mathematical models for the analysis of growth curves replicated in time are fitted and their efficiencies in a field growth study compared.

Modifications of the models necessary for their application to data where the time replication interval is greater than the recording interval are described.

The magnitude of bias in the parameters fitted because of systematic errors in the preliminary cutting of the plots, is determined. An example of the mathematical procedure is also given.

Introduction

In an earlier paper (Glenday 1955), a technique was described for the mathematical separation of the effects of natural growth and of weather from the curves of growth of a pasture in the field. In that trial 4 time replicates were used at weekly intervals and yield measurements were made weekly in each growth curve for a period of 9 weeks from closure.

The trial now reported in this journal (Brougham 1959) was designed to obtain growth curves and weather effects throughout a year by the use of the time replication technique. In the course of the work, modifications were made to the mathematical model and the technique of separation of growth and weather effects, and it is the purpose of this paper to describe these.

Methods

The field work provided 29 growth curves replicated in time at intervals of 2 weeks, and measured at weekly intervals within each for 14 weeks. There were also three replications in space.

It was first necessary to decide how to section the data so that a solution found for the first set, in the form

$$\mathbf{q} = \mathbf{Y}^{-1}\,\mathbf{b}$$

(where $\mathbf{q}$ is a column vector of the parameters, $\mathbf{Y}^{-1}$ is the inverse of the matrix of coefficients of the normal equations, and $\mathbf{b}$ is a column vector formed from the plot yields themselves) could be applied to all sets by using the common matrix $\mathbf{Y}^{-1}$ throughout.

It was decided to take sets of 4 consecutive time replicates and growth curves of 6 weeks. From such a set a weather-free curve of 0–6 weeks growth could be obtained for a stationary climate represented by the mean of 12 weeks weather. This would give growth curves

covering most grazing intervals, and 12 weeks would be a short enough period over which to determine realistic mean meteorological conditions.

Further, by using the same mathematical frame over a second growth period of 6–12 weeks is was hoped to examine the interaction of weather and stage of growth, i.e. the differences in weather effects at different stages of growth. However, the yields became much more variable with age, so that the data after 6 weeks growth were discarded, and special trials will be run to examine such interactions.

The first model fitted was that used earlier (Glenday 1955),

$$y_{i\,(j+i)} = g_i + w_{i(j+1)} + \ldots + w_{i(j+i)} + e_{i\,(j+i)}$$

where $y_{i(j+i)}$ is the yield at stage of growth i and closure date j.

g_i is the contribution from i intervals of growth (and includes the general mean). $i = 1, 2, \ldots . I$

$w_{i(j+1)} \ldots w_{i(j+i)}$ are the contributions from each of the i weather intervals during growth (there being i weather constants per equation). $j = 0, 1, \ldots . J$

$e_{i(j+1)}$ is the contribution common to all space replicates of the $i(j + i)$th treatment not explained by g_i or w_j,

the model to be fitted to the means of all space replicates in the usual way.

The fitting was carried out exactly as in the previous work, with one modification.

When the normal equations have been obtained as in Table 2 of the 1955 paper referred to earlier. the usual procedure is to eliminate one set of parameters g_i. This produces a matrix of order J but rank $(J - 1)$ so that one restriction $\Sigma\, w_j = 0$ is required to give a unique solution for w_j with zero mean. The order of matrix for inversion is then $(J - 1)$.

If this process is applied to the new set of data it is found that the rank of the resultant matrix is $(J - 2)$ not $(J - 1)$. This is because yield measurements in each curve were made at weekly intervals, while time replicates were spaced at 2-weekly intervals.

Since it was impossible to apply any further realistic restriction to the w_j to obtain a unique solution it was necessary to apply two restrictions to the normal equations, one to the g_i and one to the w_j. A logical restriction to apply would be that the highest orthogonal polynomial of the g_i

$$\phi_5 = g_1 - 5g_2 + 10g_3 - 10g_4 + 5g_5 - g_6$$

(Pearson and Hartley 1956, Table 47) should be zero. This is simple to apply by substituting the value of any g in terms of the others and deleting one equation. It will be shown later that the restriction, which ensures that any polynomial fitted to the $6g_i$ is of the 4th degree or less, is not severe, since the growth curves over the first 6 weeks are not complex in curvature.

The remainder of the work followed the previous pattern, by inverting the $(I + J - 2)$th order matrix directly or by eliminating the g_i as already described and inverting a $(J - 1)$ th order matrix to obtain the w_j, and the g_i from these by substitution in the original normal equations.

Because of the work involved in eliminating g_i, which is no longer easy to perform and does not lead to a simple $(J - 1)$th order matrix and because of the difficulty of inverting 11×11 or higher-order matrices it was decided to try an amendment to the model which would reduce the matrix for inversion to a simple one of order $(I - 1)$ (5 in this case). The new model was as follows:

$$z_{i(j+i)} = h_i + w_{i(j+i)} + e_{i(j+i)}$$

where $z_{i\ (j+i)}$ is the yield increment in the ith week for the curve with closure date j,

h_i is the growth increment for the ith week of growth irrespective of weather. $i = 1, 2, \ldots I$

$w_{i\ (j+i)}$ is the weather contribution for the $(j + i)$th week of the trial. $j = 0, 1, \ldots . J$

$e_{i(j+i)}$ is the contribution common to all replicates of the i$(j + i)$th treatment not explained by h_i or w_j, the model being fitted to the means of all space replicates.

As in the cumulative case a restriction must be applied to the h_i as well as the usual $\Sigma w_j = 0$, so that a unique solution may be obtained. However, in this method the reduced normal equations are in such a form that elimination of the w_j is simple, leaving a matrix of order $(I - 1)$ for inversion.

So that the two models would be strictly comparable the restriction applied to the h_i was

$$\phi_4 = 0 = h_2 - 4h_3 + 6h_4 - 4h_5 + h_6$$

to the 5 h_i following h_1. This is the same as the earlier restriction to the g_i.

A third fitting was also made, using the same incremental model, but using a restriction to the h_i of highest possible order to test the effect of the restriction itself on the parameters obtained. This restriction was

$$\phi_5 = 0 = h_1 - 5h_2 + 10h_3 - 10h_4 + 5h_5 - h_6$$

applied to the 6 h_i, this being equivalent to

$$\phi_6 = 0 = 6g_1 - 15g_2 + 20g_3 - 15g_4 + 6g_5 - g_6$$

applied to the 7 cumulative points $0, g_1, g_2, g_3, g_4, g_5, g_6$.

The three models were applied over several sets of the data, and the results obtained for a typical set are shown in Table 1. The set chosen is the first for which 4 complete growth curves were available, and gives Brougham's third growth curve (see Brougham 1959, Fig. 1).

The results obtained showed no essential differences in parameters fitted, or in total variation accounted for, so that from this point of view there was little to choose between cumulative and incremental models. Further, the parameters found were very little affected by the restriction applied to the h_i.

The precisions of the above estimates were compared as follows: If the parameters are determined as linear expressions of the plot yields (as in the example following) the variances of the fitted parameters will be (sum of squares of the coefficients of these plot yields) $\times$ (residual variance). This procedure gives the following standard errors.

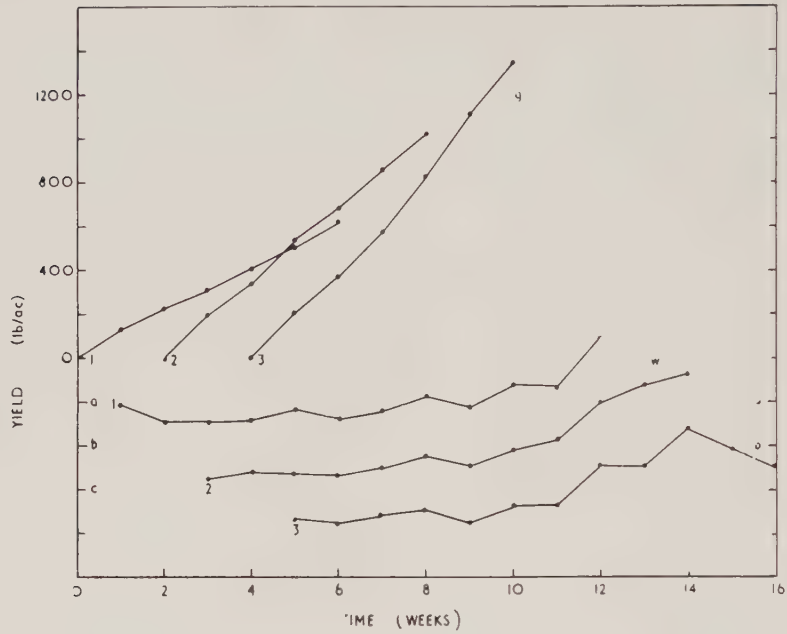


Fig. 1.—Growth (g) and weather (w) parameters for three sets of data. For clarity the three sets of (w) parameters are plotted to the same scale about a – a, b – b, c – c respectively.

TABLE 1. PARAMETERS OBTAINED BY USING THE THREE MODELS

Parameters		Original Cumulative Model $\phi^5 = 0$ on g_i	Incremental Model $\phi^5 = 0$ on g_i	Incremental Model $\phi^6 = 0$ on g_i
g or h	1	+ 128.8	+ 122.9 (122.9)	+ 126.1 (126.1)
	2	+ 225.3	+ 100.4 (223.3)	+ 97.2 (223.3)
	3	+ 306.2	+ 79.5 (302.8)	+ 82.7 (306.0)
	4	+ 413.9	+ 106.6 (409.4)	+ 103.4 (409.4)
	5	+ 538.5	+ 125.3 (534.7)	+ 128.5 (537.9)
	6	+ 617.7	+ 79.0 (613.7)	+ 75.8 (613.7)
w	1	— 19.8	— 13.9	— 17.1
	2	— 76.3	— 95.4	— 92.2
	3	— 96.5	— 85.2	— 88.4
	4	— 90.1	— 86.5	— 83.3
	5	— 32.4	— 33.6	— 36.8
	6	— 83.2	— 82.3	— 79.1
	7	— 46.5	— 38.9	— 42.1
	8	+ 18.5	+ 17.7	+ 20.9
	9	— 12.0	— 24.4	— 27.6
	10	+ 72.0	+ 72.7	+ 75.9
	11	+ 65.5	+ 68.7	+ 65.5
	12	+ 300.8	+ 301.0	+ 304.2
Residual variance		660.0	516.1	516.1

The h_i are given cumulatively in brackets.

TABLE 2. STANDARD ERRORS OF PARAMETERS (as decimals of residual SE)

Parameters		Original Cumulative Model $\phi_5 = 0$	Incremental Model $\phi_5 = 0$	Incremental Model $\phi_6 = 0$
g or h	1	0.819	0.672	0.638
	2	0.557	0.618	0.554
	3	0.553	0.497	0.446
	4	0.668	0.412	0.446
	5	0.799	0.502	0.554
	6	0.626	0.618	0.638
w	1	1.292	1.134	1.114
	2	1.422	1.102	1.068
	3	0.942	0.781	0.750
	4	0.992	0.732	0.716
	5	0.783	0.605	0.595
	6	0.824	0.600	0.595
	7	0.799	0.605	0.595
	8	0.869	0.600	0.595
	9	0.974	0.714	0.716
	10	1.052	0.732	0.750
	11	1.401	1.042	1.068
	12	1.585	1.102	1.114

Again the differences are not great. However, because growth curves would be obtained from the second and third methods by cumulative addition of the h_i determined, it is clear that the latter (incremental) methods determine the w_j rather more accurately at the expense of accuracy in the g_i. This is a consequence of the alteration in the model, since it will be remembered that in the last two methods the number of w parameters used in each prediction equation was reduced to unity.

In view of the above examination it was decided to complete the work using the third method; that is, the method giving the best precision to the w_j, the method with the least stringent restriction applied to the g_i, and finally that requiring least matrix inversion.

The chosen procedure will now be demonstrated using generalised data for 4 growth curves, viz.

YIELD INCREMENTS

Closure date	Weeks of Growth 1	2	3	4	5	6
0	a	b	c	d	e	f
2	g	h	i	j	k	l
4	m	n	o	p	q	r
6	s	t	u	v	w	x

Then the normal equations are

| | | |

$$
\begin{array}{cccccccccccccccc|c|c|c|c}
 & & & & & & & & & & & & & & & & & & & \\
 & & & & & & & & & & & & & & & & & & & \\
 & & & & & & & & & & & & & & & & & & & \\

When this is expanded so that the h_i are given as linear expressions of the increments a to x we get

$$\mathbf{q}' = \mathbf{a}' \cdot \frac{1}{10080} \begin{vmatrix} 840 & 840 & 840 & 840 & 840 \\ 840 & 840 & 840 & 840 & 840 \\ 921 & 1479 & 921 & 327 & 921 \\ -1437 & 669 & 1731 & 669 & 579 \\ 282 & -762 & 282 & 2694 & 282 \\ -834 & -222 & -258 & -222 & 3198 \\ 81 & -639 & -81 & 513 & -81 \\ 2277 & 171 & -891 & 171 & 261 \\ 780 & 1860 & 780 & -1020 & 780 \\ -1020 & 780 & 1860 & 780 & -1020 \\ 282 & -762 & 282 & 2694 & 282 \\ -834 & -222 & -258 & -222 & 3198 \\ -222 & -258 & -222 & -834 & -222 \\ 2694 & 282 & -762 & 282 & -1338 \\ 780 & 1860 & 780 & -1020 & 780 \\ -1020 & 780 & 1860 & 780 & -1020 \\ 171 & -891 & 171 & 2277 & 171 \\ 513 & -81 & -639 & -81 & 2529 \\ -222 & -258 & -222 & -834 & -222 \\ 2694 & 282 & -762 & 282 & -1338 \\ 669 & 1731 & 669 & -1437 & 669 \\ 327 & 921 & 1479 & 921 & -1689 \\ 840 & 840 & 840 & 840 & 840 \\ 840 & 840 & 840 & 840 & 840 \end{vmatrix}$$

i.e. $\mathbf{q}' = \mathbf{a}'\,\mathbf{P}'$ where $\mathbf{P} = \mathbf{Y}^{-1}\,\mathbf{K}$

Then h_1 is found from the restriction $\phi_5 = 0$ and w_j are found from the normal equations, with the check that $\Sigma\, w_j = 0$.

The sum of squares accounted for by the fitting process is found as the sum of products of the 18 parameters fitted and the 18 terms at the right-hand side of the normal equations. From this calculation the residual variance is found in the usual way, and the variances of the parameters themselves as the products of the residual variance and the sums of squares of the appropriate columns of coefficients in $\mathbf{P}'$.

It is now easy to operate on any section of the data with this general solution. In practice the solution was applied to the data at 2-weekly intervals, the first using curves 1–4, the second 2–5, and so on. The solution for the third set, the subject of the example here, is given in Fig. 1, together with the succeeding two sets to show how the weather fluctuations repeat themselves in each set of 12 w_j.

For the example the residual variance was 516.1, so that the SEs of the h_i averaged $\pm$ 12.4, while the SEs of the 8 centre w_j were $\pm$ 15.1. For the whole analysis (Brougham 1959, Figs. 1 and 4) the mean SEs of the h_i rose from $\pm$ 15.0 for the first 4 sets to $\pm$ 95.4 for the middle 4 sets, falling again to $\pm$ 33.7 for the final 4 sets, with proportional changes in the SEs of the w_j.

Each set of g and w parameters obtained is for constant weather over 12 weeks. Since the generalised increment for the ith week of growth is estimated from data on the ith week and data 2, 4, and 6 weeks later, the date on which this increment would be obtained under a smoothly changing season can be taken as week $(i + 3)$.

Also, because the parameters are obtained for constant weather over 12 weeks, the trends in growth owing to gradual changes in season appear as trends in the w parameters, and in the earlier work (Brougham 1955) these trends were themselves separated and added to the g parameters to give theoretical curves of growth for smoothly changing climate.

In the present data, in which overlapping sets of w parameters were generated, a continuous set free of seasonal trends can be obtained as follows. Each set of 12 w_j (about zero mean) is reduced to first differences, the end 2 pairs of each set omitted leaving only the central 8 shown in Table 2 to be the most accurate, and the continuous set of w_j obtained as means of the resulting 4 estimates of each first difference, with the addition of a constant to give a zero mean. This final set of w parameters can then be used to examine relationships between short-term weather changes and responses in growth.

In the field work, there is one form of error which is hard to avoid because of the difficulty of making the preliminary cut accurately. Some of the herbage will be rolled and not cut, and this means that in the data obtained the first increment recorded in particular growth curves may be too high. The sharp bend in some of the early curves at one week's growth may be a result of this.

From the equations giving the parameters in terms of the plot increments $\mathbf{q}' = \mathbf{a}'\,\mathbf{P}'$ it is easy to calculate the effects on all parameters of such systematic error. It is found that if, a, g, m, and s are all increased by 32 units, the changes in the parameters are

h_1	+ 31	w_1	+ 1
h_2	+ 1	w_2	— 1
h_3	— 1	w_3	+ 1
h_4	+ 1	w_4	— 1
h_5	— 1	w_5	+ 1
h_6	+ 1	w_6	— 1
		w_7	+ 1
		w_8	— 1
		w_9	+ 1
		w_{10}	— 1
		w_{11}	+ 1
		w_{12}	— 1

Thus any such error affects the parameter h_1 mainly, with only minor effects on the remainder. Since the effects of such an error would be simply to lower the base-line against which the growth curves are plotted, the growth curves given by Brougham show the increments for the first week as dotted lines.

REFERENCES

Brougham, R. W. 1955: A Study in Rate of Pasture Growth. *Aust. J. agric. Res. 6:* 804-12.

————1959: The Effects of Season and Weather on the Growth Rate of a Ryegrass-Clover Pasture. *N.Z. J. agric. Res. 2:* 283-96.

Glenday, A.C. 1955: The Mathematical Separation of Plant and Weather Effects in Field Growth Studies. *Aust. J. agric. Res. 6:* 813-22.

Pearson, E. S.; Hartley, H. O. 1956: "Biometrika Tables for Statisticians", Vol. 1. C.U.P.

SEED DISINFECTION

XV. GLASSHOUSE TESTS FOR CONTROL OF DAMPING-OFF OF PEA SEED

By H. Jacks, Massey Agricultural College, Palmerston North

(*Received for publication, 28 October 1958*)

Summary

The effect was investigated of eleven seed dressings on the prevention of damping-off of pea seeds grown in soil inoculated with *Pythium ultimum* Trow.

Eleven varieties of pea seeds were used in these tests, and overall the most successful treatments proved to be those based on dichlone and captan. These treatments also gave a significant reduction in the percentages of post-emergence infection of seedlings.

Successful treatments accelerated the speed of emergence of seedlings.

Introduction

Damping-off of pea seed is common in moist soil, and *Pythium* species are usually isolated either from seeds that failed to germinate or from post-emergence lesions on seedlings. In previous tests (Jacks 1951) only one pea variety was used and information was sought on the effectiveness of seed dressings in preventing damping-off of other varieties. The experiments reported here were carried out in inoculated soil maintained at 50% water-holding capacity, a relatively high moisture content.

Materials and Methods

The germination capacity of pea seed was assessed in moist sand at 68 ± 4°F. Germination percentages given in parentheses were determined on 300 seeds of the following varieties: Blue Bantam (88%), Greenfeast (68%), Little Marvel (72%), Lord Chancellor (87%), Meteor (100%), Onward (78%), Stratagem (86%), Thomas Laxton (96%), William Massey (87%), Witham Wonder (83%), and Yorkshire Hero (63%).

The following seed dressings were applied at 0.25% of seed weight:

Captan: 75% (Orthocide 75);

Copper compounds: copper carbonate 95%, copper oxychloride 50% (Cuprox), cuprous oxide 90% (Red Cuprocide);

Dithiocarbamates: ferbam 70% (Fermspray), thiram 70% (Thirodust), zineb 65% (Dithane Z 78);

Organo mercury compounds: methyl mercuric cyanoguanidine 2.2% (Panogen 15), phenyl mercury chloride 1% (Leytosan);

Quinones: chloranil 98% (Spergon), dichlone 98% (Phygon).

TABLE 1. EFFECT OF SEED DRESSINGS ON EMERGENCE OF PEA SEEDS IN SOIL INOCULATED WITH *P. ultimum*

Treatment	B. Bantam		Greenfeast		L. Marvel		L. Chancellor		Meteor		Onward		Stratagem		T. Laxton		W. Massey		W. Wonder		Y. Hero	
	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle
Check	1	2	18	25	1	2	2	5	21	27	0	0	3	9	29	32	17	24	0	0	0	0
Captan	84	67	96	80	48	44	79	63	100	86	61	51	84	67	96	82	72	59	58	50	43	41
Copper carbonate	59	50	77	65	5	8	45	42	87	69	20	25	50	45	38	37	62	52	38	38	15	19
Copper oxy-chloride	41	40	80	65	24	29	54	47	90	72	37	37	37	37	60	51	52	46	42	40	5	11
Cuprous oxide	20	26	45	42	2	4	40	39	68	56	7	14	21	27	35	37	32	33	5	11	0	0
Ferbam	5	11	39	38	3	9	30	32	81	65	14	21	27	30	72	58	8	15	0	0	3	7
Thiram	83	66	66	54	18	25	47	42	90	75	31	33	37	37	86	69	39	39	33	35	13	18
Zineb	45	42	84	68	8	16	49	44	87	69	34	35	28	31	90	73	40	39	17	24	4	9
Methyl mercuric cyano-guanidine	3	7	35	36	1	4	3	7	31	33	1	2	7	15	67	54	5	11	0	0	0	0
Phenyl mercury chloride	9	16	17	23	4	9	29	33	69	56	1	4	7	14	82	65	42	40	1	2	0	0
Chloranil	23	28	59	50	6	11	26	30	69	57	8	15	28	31	85	68	27	31	4	8	10	17
Dichlone	73	59	99	82	49	44	78	62	92	74	66	55	66	55	98	84	85	68	75	61	44	41
Sig. diff. at 1%		13		18		14		17		14		15		12		16		14		13		18

TABLE 2. EFFECT OF SEED DRESSINGS ON POST-EMERGENCE INFECTION OF PEA SEEDLINGS GROWN IN SOIL INOCULATED WITH *P. ultimum*

Treatment	B. Bantam		Greenfeast		L. Marvel		L. Chancellor		Meteor		Onward		Stratagem		T. Laxton		W. Massey		W. Wonder		Y. Hero	
	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle	%	Equiv. Angle
Check	100	90	35	37	100	90	100	90	68	56	—	—	100	90	61	52	62	53	—	—	—	—
Captan	17	24	4	12	45	41	31	33	19	26	75	60	27	32	12	21	26	31	37	38	50	45
Copper carbonate	49	45	25	31	100	90	71	58	36	37	82	64	65	54	70	58	30	34	87	68	95	77
Copper oxychloride	82	65	50	45	93	74	57	49	32	35	96	79	77	61	37	38	30	34	97	80	100	90
Cuprous oxide	92	73	39	39	100	90	38	39	37	38	71	58	93	76	36	37	68	56	89	71	—	—
Ferbam	100	90	57	50	100	90	59	50	95	78	92	74	92	75	38	38	94	76	—	-	100	90
Thiram	16	25	38	39	89	69	46	43	19	26	92	77	77	62	34	35	44	41	86	68	76	61
Zineb	80	65	31	34	100	90	42	41	27	31	66	56	74	60	29	32	37	38	91	73	83	66
Methyl mercuric cyanoguanidine	100	90	67	55	100	90	80	62	87	69	100	90	100	90	42	41	90	71	—	—	—	—
Phenyl mercury chloride	81	64	90	73	100	90	3	10	62	53	100	90	97	80	41	40	34	36	100	90	—	—
Chloranil	93	76	30	34	100	90	88	70	62	52	89	71	68	56	35	36	52	47	100	90	100	90
Dichlone	24	30	19	26	55	48	22	29	9	18	53	47	46	43	21	28	8	17	30	33	44	42
Sig. diff. at 1%		25		17		16		24		24		33		25		15		18		23		22

Nursery soil based on yellow-grey loam was prepared by the method given earlier (Jacks 1951), and maintained at a moisture level of 50% water holding capacity throughout the tests. Soil was inoculated with *Pythium ultimum* Trow. by mixing steam-disinfected soil and 4-day-old sand-barleymeal cultures. Emergence tests were carried out in tins, each holding 1 kg of soil. Five replications each of 40 seeds sown at 1 in. depth were used for each treatment. Glasshouse temperature throughout the tests was 68 ± 8°F. Five weeks after sowing, records were made of total emergence and of seedlings showing sypmtoms of post-emergence damping-off. The latter included plants showing minor lesions as well as those plants which had died from damping-off. Isolations from diseased tissues proved *Pythium* as the causal organism.

For the assessment of speed of emergence, additional replicates were used, viz: seedlings were counted and removed every 48 hours throughout the test. The speed of emergence was calculated by the formula:

$$\text{Velocity coefficient (VC)} = \frac{\text{Total emergence}}{\text{sum } (N_1 \times \overline{D}_1) + (N_2 \times D_2) + \text{etc.}} \times 100$$

in which N is the number of seedlings emerged at the date of recording and D the number of days from the date of sowing when emergence was recorded.

TABLE 3. EFFECT OF SEED DRESSINGS ON SPEED OF EMERGENCE (IN TERMS OF VELOCITY COEFFICIENT) OF PEA SEEDLINGS GROWN IN SOIL INOCULATED WITH *P. ultimum*

Treatment	B. Bantam	Greenfeast	L. Marvel	L. Chancellor	Meteor	Onward	Stratagem	T. Laxton	W. Massey	W. Wonder	Y. Hero
Check	12	14	12	10	14	—	11	12	15	—	—
Captan	24	24	22	24	22	24	24	25	22	22	20
Copper carbonate	19	23	13	20	16	13	18	16	20	16	15
Copper oxychloride	16	21	17	21	20	15	16	18	17	16	9
Cuprous oxide	13	19	10	17	16	10	18	17	18	10	—
Ferbam	12	17	9	16	17	14	16	20	11	—	10
Thiram	22	20	16	20	22	14	18	22	19	14	14
Zineb	19	23	16	18	18	17	17	23	18	13	11
Methyl mercuric cyanoguanidine	12	17	11	11	14	11	14	20	12	—	—
Phenyl mercury chloride	11	18	12	17	15	10	15	20	19	9	—
Chloranil	14	15	13	17	18	13	16	21	17	14	13
Dichlone	24	24	20	24	20	21	24	24	23	24	23

Results

The total emergence percentages given in Table 1 were calculated on the basis of the germination capacity of the respective varieties used. All figures are given to the nearest whole number. Dichlone and captan gave the greatest protection, with significantly higher total emergence percentages generally, although most treatments showed improved total emergence percentages over untreated controls. Differential responses of varieties to treatments were observed, the highest emergence percentages with all treatments being given by the varieties Meteor, Greenfeast, T. Laxton and W. Massey.

The percentages of diseased seedlings (Table 2) were calculated on the basis of the number of seeds that germinated (Table 1). Owing to the severe conditions of the test a high percentage of seedlings showed post-emergence lesions; however, a large proportion of these lesions was smaller in extent than those in the controls. Therapeutant treatments giving the highest emergence percentages also gave the greatest protection against post-emergence damping-off.

The therapeutants used, in general, accelerated the speed of emergence over that of the controls (Table 3), and thus allowed a greater number of seedlings to escape strangulation by the pathogen. The highest velocity coefficient of emergence was given by the most successful treatments, and with these greatest seedling vigour was also observed.

Discussion

These tests demonstrated that the seed treatments (Jacks 1956) can inhibit soil organisms in direct contact with the seed. How far this inhibitory effect can extend through the soil from the immediate neighbourhood of the treated seeds has not been assessed but such action can be assumed because of the reduction in the numbers of diseased seedlings. The dispersibility of the active material in a seed dressing varies with the type of formulation, and its prophylactic zone around the seed varies with the soil texture, organic matter content, and moisture status.

A proportion of more slowly germinating seeds in a sample may not depress the average speed of emergence owing to their failure to emerge at all as a result of attack by the soil organisms. The use of the velocity coefficient as a means for rapid evaluation of seed dressings would, therefore, involve close comparisons with figures for total emergence.

Increased speed of germination and seedling vigour brought about by some treatments ensured greater percentages of surviving plants. Although the less vigorous varieties responded most to treatment, results suggest that the more vigorous seeds would be more suitable for growing under adverse field conditions.

CONCLUSION

Dichlone and captan, as seed treatments, applied at 0.25% of seed weight, increased the emergence and vigour of a number of pea varieties in moist soil infected with *Pythium*. The most vigorous varieties were Meteor, Greenfeast, Thomas Laxton, and William Massey, a factor of importance for the choice of varieties.

REFERENCES

Jacks, H. 1951: Seed Disinfection. I. Preliminary Selection of Vegetable Seed Protectants. *N.Z. J. Sci. Tech. A22(3):* 27-38.

————1956: Important Factors in Seed Disinfection. *N.Z. Sci. Rev. 14(9):* 119-21.

UNINTENTIONAL INTRODUCTION OF PLANTS INTO NEW ZEALAND

By A. J. Healy, Botany Division, Department of Scientific and Industrial Research, Christchurch

(Received for publication, 4 September 1958)

Unintentional or incidental introduction of weed species into New Zealand has been, and still is effected by diverse means, several of which have been reported by botanists from 1786 to the present time.

This note is concerned with three examples of such introduction, and the significance of the particular means of introduction.

The first example is a straw envelope off a whisky bottle, picked up from a rubbish heap on a North Canterbury farm. Fig. 1 shows the envelope cut and flattened out, with a flowering stem of bindweed (*Calystegia sepium* (L.) Roem. et Schult.) on extreme right. Fig. 2 shows portions of inflorescences of tall oat grass (*Arrhenatherum elatius* (L.) J. et C. Presl) and a bent grass (*Agrostis* sp.), together with the flowering stem of bindweed, all dissected out from the straw of the cover.

Apart from the circumstances that the bindweed was in the non-seeding stage, and that one, and possibly both of the grasses already occur in New Zealand, the important features are—(i) that such envelopes provide an entry for

Fig. 1

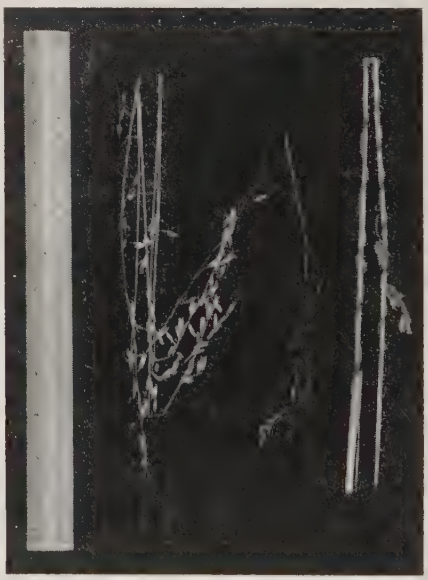


Fig. 2

Fig. 1.—Flattened-out straw envelope off whisky bottle, showing flowering stem of bindweed (*Calystegia sepium*) on extreme right.

Photo: A. L. Bryant.

Fig. 2.—Flowering and fruiting material dissected from straw envelope. Left: tall oat grass (*Arrhenatherum elatius*); centre: bent grass (*Agrostis* sp.); right: bindweed (*Calystegia sepium*).

Photo: A. L. Bryant.

plants into New Zealand; (ii) that such containers when discarded are generally thrown on rubbish heaps and other suitable sites for weed establishment; and (iii) that some fruits, at least, of the two grasses were viable.

The second instance was that of a plate-glass container from an English source, examined in the Wellington district. This crate contained a quantity of wheaten straw (Fig. 3) in which was found large non-flowering portions of Californian thistle (*Cirsium arvense* (L.) Scop.), cleavers (*Galium aparine* L.) willow weed (*Polygonum* cf. *lapathifolium* L.), with ripe fruiting material of *Beta* sp., nipplewort (*Lapsana communis* L.) and a hemp nettle (*Galeopsis* sp.).

When examined, this straw was exposed to the wind adjacent to a vacant area of waste land, and information was given that the straw was used locally for compost heaps and garden purposes. I noted also that it was being used as crate material in racing-pigeon cages, which cages are dispersed through the country for sporting purposes: these cages are, incidentally, usually disposed about railway stations, etc., where suitable conditions for weed establishment exist.

The third instance is introduction of fruits and seeds in turn-up cuffs of trousers. The fruits (Fig. 4) had lodged in cuffs of a traveller's clothes during a trip through Spain, southern France, Switzerland, and Italy; the quantity and variety would no doubt have been greater had the travelling been done later in the fruiting season.

Fig. 3

Fig. 4

Fig. 3.—Glass crate containing wheaten straw, showing amount of straw introduced in a single container.

Photo: B. Lawson.

Fig. 4.—Plant material from trouser-cuffs. Left: fruits and seeds; right: chaff and dross.

Photo: A. L. Bryant.

The fruits (not all identified) were mainly representative of the grass family (*Gramineae*), with a smaller number from the daisy family (*Compositae*), and a spore-bearing fragment of a fern frond.

This unintentional collection of fruits by tourists could be responsible for introduction of serious weeds into New Zealand, this being especially so with returning parties of farmers. The turning out and cleaning of tourists' trouser-cuffs prior to return to New Zealand, or burning of the material if removed on arrival would be in the interests of agriculture. The throwing out of trouser-cuff debris on the rubbish heap or bare garden might result in a crop of weeds.

My thanks are due to Mr. I. A. McNeur, Botany Division, Department of Scientific and Industrial Research, Wellington, for retention of his trouser-cuffs "harvest" for examination and to Messrs A. L. Bryant and B. Lawson, Animal Research Station, Wallaceville, for the photographs used.

RUMEN NITRATE METABOLISM AND THE CHANGES OCCURRING IN THE COMPOSITION OF THE RUMEN VOLATILE FATTY ACIDS OF GRAZING SHEEP

By N. D. Jamieson, Animal Research Station, Department of Agriculture, Wallaceville

(*Received for publication, 16 October 1958*)

Summary

Adult sheep and weaned lambs grazing ryegrass and clover pasture, and sampled at approximately monthly intervals, exhibited marked variations in the molecular percentage composition of the individual rumen volatile fatty acids. These variations were characterised by comparatively large changes in the percentage of acetic acid and compensatory changes in an inverse direction in the percentages of propionic and, to a smaller extent, n-butyric and the branched-chain acids.

It has been found that similar changes in the relative amounts of acetic, propionic, and to a less extent n-butyric acids, can be induced by the oral administration of nitrate or its reduction product nitrite to sheep on pasture. Two factors may be operating in normal grazing sheep to alter the composition of the volatile fatty acids of the rumen; one the known variable nitrate content and the other the protein content of the pasture.

It is postulated that a lowered molecular percentage of acetic acid may be due to a lack of acetic acid precursors usually present in the rumen; these probably being soluble carbohydrate or plant organic acids. Such a deficiency might be absolute, or brought on by the competitive removal of these substrates in their function as hydrogen donors required to bring about the reduction of nitrate to ammonia. Such conditions can apparently occur during periods of high protein levels in the pasture.

Introduction

A survey of the levels of rumen total volatile fatty acids and their molecular percentage composition, in samples taken from adult sheep and weaned lambs grazing the same pasture throughout the year, showed large fluctuations in the relative content of the constituent acids. This fluctuation consisted of a differential movement between the molecular percentages of acetic acid and those of the other volatile fatty acids. A lowered level of acetic acid can occur in the rumen of the normal animal during various times of the year owing to a marked fall in the molecular percentage of acetic acid in the total volatile fatty acid fraction, rather than to a fall in the level of the total volatile fatty acids. Observations, as yet unpublished, showed that such periods of lowered acetic acid concentration in the rumen appeared to be linked with low positive and negative increments in the rate of change in live weight over the time of sampling, and occurred more frequently during autumn and spring.

In the course of experiments involving the administration of nitrate to sheep on pasture it was found that the treatment resulted in a marked

change in the rumen fermentation such that the molecular percentage of acetic acid fell, while the percentages of propionic and to a lesser extent butyric acids showed a concomitant rise.

In view of these findings, it became of direct interest to examine the effect of other possible nitrate reduction products on the molecular percentage composition of the rumen volatile fatty acids. The compounds investigated were: nitrate, nitrite, hydroxylamine, and hydrazine.

The nitrate ion forms a normal component of fresh pasture, and is known to be reduced in the rumen to nitrite (Sapiro *et al.* 1949). Lewis (1951a, b) has shown that both nitrate and nitrite are ultimately reduced to ammonia and that hydroxylamine might form a possible intermediate in the reduction sequence, since this compound can be reduced by washed suspensions of rumen micro-organisms to ammonia.

These changes have been postulated in the wider field of nitrate metabolism in plants and micro-organisms involved in the processes of nitrification, denitrification, nitrate assimilation, and nitrogen fixation. The metabolic pathway of nitrate reduction relevant to the rumen, may be outlined thus—
nitrate → nitrite → (hyponitrite) → hydroxylamine → ammonia. The evidence for the occurrence of intermediates between nitrite and ammonia is largely circumstantial owing to the chemical lability and toxicity of compounds like hyponitrous acid and hydroxylamine. Free amino-acids in the presence of nitrite could undergo deamination with the production of nitrogen and the decomposition of the nitrite. Condensations may occur between hydroxylamine and keto-acids to form oximes which under the conditions prevailing in the rumen might undergo reduction to amino-acids. Holtenius (1957) investigated the metabolism of nitrate and nitrite in the normally functioning rumen and obtained evidence supporting the view that nitrate reduction products were to some extent involved in these side reactions.

METHODS

Analytical

The methods used to determine pH, E_h, ammonia-nitrogen and total volatile fatty acids, were as given previously (Jamieson 1958b). The molecular percentages of the individual fatty acids in the total volatile fatty acid distillates were obtained by isolation of their mixed sodium salts followed by a quantitative separation using the method of gas liquid chromatography (James and Martin 1952).

Protein-nitrogen levels quoted in Table 1 represent the difference between total nitrogen and non-protein nitrogen determinations by a semi-micro Kjeldahl method using the Wagner-Parnass apparatus.

Sheep

All the animals used in this work were Romney × Southdown crossbred sheep and were maintained under as near normal farm conditions as possible on a ryegrass/clover pasture throughout the year.

The sheep used in the dosing experiments were one to two years old and their live weights fell in the 50–100 lb range. All rumen samples

were obtained by rumen intubation. The materials dosed were administered as five per cent aqueous solutions directly into the rumen by means of a polythene tube and funnel which were rinsed with a similar volume of water. The following materials were used: nitrate, given as potassium nitrate "Analar"; nitrite as sodium nitrite "Analar"; hydroxylamine, in the form of hydrochloride; and hydrazine as the sulphate. Since the maximal safe dose of sodium nitrite for a 100 lb sheep is about 4 g, the hydroxylamine and hydrazine compounds were given in amounts not exceeding the theoretical conversion of the above amount of nitrite.

Results

Normal Sheep on Pasture

Rumen-liquor samples from groups of weaned lambs and adult sheep grazing the same field of ryegrass/clover pasture, obtained at approximately monthly intervals over the course of three seasons, have been analysed for total volatile fatty acids, ammonia-nitrogen, protein-nitrogen, and pH. The molecular percentages of the individual acids, from formic to n-valeric acid, were determined on combined samples from the adults and weaned lambs by gas liquid chromatography. The results briefly summarised in Table 1 illustrate the wide variation in molecular percentages of the individual volatile fatty acids occurring in sheep on pasture.

These differential changes in the molecular proportion of acetic acid relative to the other acids of the fraction appear to be due mainly to changes in pasture composition. At any given sampling time, very similar results were obtained from both lambs and adult sheep on the same pasture. Samples showing low percentages of acetic acid exhibited a seasonal fluctuation, tending to occur more frequently in the spring and autumn. Animals whose rumen-liquor samples showed a low percentage of acetic acid tended to be making poorer growth than that occurring when the molecular percentage of acetic acid in the rumen liquor was high. Weight changes in sheep, when expressed as lb/100 lb live weight, were very similar in both weaned lambs and adult sheep on the same pasture.

The values quoted in Table 1 illustrate the following points: The pH values of the rumen contents were normal. The total volatile fatty acids cover the accepted normal range, and were determined on individual sheep within the groups. Occasionally low values were obtained and were probably due to low intake, fasting, or transient ruminal upset in individual animals. The total volatile fatty acids level appeared to be quite independent of any changes in the molecular percentage of acetic or other constituent acids.

Ammonia-nitrogen values showed a highly significant (at 0.1%) correlation with the concentration in mM/100 ml rumen liquor of the combined branched-chain acids (i-butyric, i-valeric, and α-methylbutyric acids). The protein-nitrogen values also showed a correlation significant at the 1.0% level with these combined branched-chain acids. These findings are in agreement with those of el-Shazly (1952a) who concluded that iso-butyric acid and the branched-chain C_5 acids arise from microbial attack on protein in the rumen.

TABLE 1. RUMEN LIQUOR FROM APPARENTLY NORMAL SHEEP ON RYEGRASS/CLOVER PASTURE

	pH Rumen Contents	Protein-N mg/100 ml	Ammonia-N mg/100 ml	Total V.F.A. mM/100 ml	Molecular Percentages of Individual Fatty Acids							
					Formic	Acetic	Propionic	i-Butyric	n-Butyric	i-Valeric	α-Methyl-butyric	n-Valeric
Mean values of 47 samplings	6.6	303	37	9.0	0.4	53	23.5	3	15	2	1.5	1.5
Range of values	5.7–7.5	112–382	15–75	3.2–15.3	0–2.0	38–64.5	15–30	1.0–4.5	9.5–22	0.5–4.0	0–3.5	0.5–3.0
Sample showing highest % acetic acid	6.3	300	36	11.4	0	64.5	18	2	12.5	1	1	1
Sample showing lowest % acetic acid	6.3	380	69	8.8	1.0	38	29	3.5	21	3	3	1.5

TABLE 2. RUMEN V.F.A. OF SHEEP DOSED WITH NITRATE ON RYEGRASS/CLOVER PASTURE

Animals and Treatment	Sampling Time	pH Rumen Contents	Total V.F.A. mM/100 ml	Individual V.F.A. Molecular Percentages							
				Formic	Acetic	Pro-pionic	i-Butyric	n-Butyric	i-Valeric	α-Methyl-butyric	n-Valeric
Mean values for 2 sheep of live-weight 65 and 72 lb, dosed 8 g KNO_3 thrice daily for three days.	Pre-dosing	6.4	12.8	0	63	20	2	11	1.5	1.5	1.5
	24 h after last dose	6.7	7.6	0	48	32	1.5	14	1.5	1.5	1.5
Single dose of 25 g KNO_3 to an 80 lb sheep A.	Pre-dosing	6.6	10.4	0	67	19	2	10	1	0.5	1
	8 h post-dosing	6.7	10.3	0	69	18	1.5	8.5	1	1	1
	24 h post-dosing	6.5	9.2	*	51	31	2	13	1.5	0.5	1.5
Single dose of 20 g KNO_3 to a 53 lb sheep B.	Pre-dosing	6.9	6.7	0	58	21	3	13	2	2	2
	8 h post-dosing	7.2	3.0	0	69	19	2	8	1	1	1
	24 h post-dosing	6.8	6.25	0	48	33	2.5	13.5	1	1	1

* In this and subsequent tables, denotes that the acid concerned was present and identifiable on the chromatogram in amount less than 0.5 molecular percentage.

Formic acid was not generally detectable in the total volatile fatty acids of rumen liquor samples from sheep on pasture, but became so and showed small increments in its molecular percentage as the molecular percentage of acetic acid fell.

Nitrate is a known normal constituent of herbage, and the pasture on which these animals were grazing has been shown to contain fluctuating amounts of nitrate—with a norm of about 1% potassium nitrate on the dry matter (Jamieson 1958b).

Sheep Dosed Nitrate

The upper section of Table 2 records the first observation of a change in composition in the total volatile fatty acids after thrice-daily dosing of potassium nitrate for three days.

The similar effect produced by a larger single dose of potassium nitrate on two sheep is shown in the lower section of Table 2. The principal effect appears to be a fall in the percentage of acetic acid with concomitant rises in the percentages of propionic, and to a lesser extent n-butyric, acids. Eight hours after a single dose of nitrate, the percentage of acetic acid showed a rise, while percentages of n-butyric and to a lesser degree propionic acids fell. Sheep B which showed this effect most clearly also showed a marked fall in the value for total volatile fatty acids, while the E_h values (not quoted) showed a sharp increase towards more positive values. This transient rise in the molecular percentage of acetic acid in the rumen liquor was also shown by animals 14 and 18 in Table 3, and was apparently due to a temporary upset in the rumen fermentation.

It will be noted that there has been no change in the concentration of the branched-chain fatty acids and n-valeric acid.

Changes in the molecular composition of the rumen volatile fatty acids of sheep dosed potassium nitrate daily for fourteen days are shown in Table 3.

Control Animals. During these experiments, analyses were made on samples from sheep of similar age and liveweight grazing the same pasture as the dosed sheep. The relative stability of the molecular percentage composition is illustrated by the values of the standard deviations from the mean of a set of samples taken from different normal animals at the beginning of the experiment and the figures for the standard deviation in the ten samplings made on the two control sheep during the experiment.

Dosing Schedule. Each animal was given 25 g potassium nitrate after the preliminary sample on the first day, and again on the second day. Deaths from nitrate poisoning occurred on the third day and the sheep were not dosed. The dose was lowered to 10 g for the fourth to eighth days; increased to 15 g on the ninth, 25 g on the tenth, and lowered slightly to 20 g on the eleventh and 18 g on the twelfth to fourteenth days inclusive. The aim was to administer as high a nitrate dose as possible without deaths occurring from acute toxicity.

TABLE 3. RUMEN V.F.A. OF GRAZING SHEEP DOSED FOR 14 DAYS WITH NITRATE
(First dose after sampling on 1st day; last dose on 14th day)

Animals and Treatment	Sample-	Day	pH Rumen Contents	Total V.F.A. MM/100 ml	Individual Volatile Fatty Acids				Molecular Percentages			
					Formic	Acetic	Pro-pionic	i-Butyric	n-Butyric	i-Valeric	α-Methyl-butyric	n-Valeric
Mean values for 2 control sheep on same pasture as dosed animals. Mean l.w. 73 lb		1	6.4	9.15	0	56	25	2.5	13	1.5	1.5	1.5
		5	6.6	7.6	0	57	23	2	14	1.5	1.5	1.5
		8	6.7	7.0	0	60	21	2	13	1	1.5	1.5
		12	6.7	7.0	0	59	22	2.5	13	1.5	1	1
		17	6.8	6.2	0	56	23	2.5	14	2	1.5	1.5
		24	6.9	7.4	0	57	23	2.5	13	1.5	1	2
S.E. of Means:												
7 normal sheep		1	± 0.22	± 0.93		± 2.42	± 1.15	± 0.32	± 1.47	± 0.27	± 0.32	± 0.13
2 controls (10 det.)		5-24	± 0.16	± 1.23	—	± 2.62	± 1.20	± 0.43	± 1.55	± 0.55	± 0.46	± 0.33
Sheep 18, l.w. 78.5 lb	Pre-dosing	1	6.4	9.6	0	57	25	2.5	12.5	1	1.5	1.5
		3	7.3	4.15	0	70	17	4.5	3	2	2.5	1
		5	6.7	5.1	1	45	34	2.5	14	1	0.5	1.5
Died on 12th day		8	6.5	6.85	0	54	29	2	12.5	1	1	1.5
Sheep 14, l.w. 72 lb	Pre-dosing	1	6.0	11.1	0	59	25	2	11	1.5	1	1.5
		3	6.6	4.0	0	85	14	*	1	0	0	0
		5	6.1	8.6	2	45	34	1	15	0.5	0.5	2
		8	6.7	7.6	0	51	33	3	8.5	1	1	2
		12	6.3	8.25	*	43	33	3	15.5	1.5	1.5	3
	Post-dosing	17	6.6	8.15	0	41	33	3	19	1.5	1	2
		24	6.6	8.1	0	55	26	2.5	13	1.5	1	1.5
Sheep 16, l.w. 62 lb	Pre-dosing	1	6.3	9.15	0	57	25	3	11	1.5	1.5	1.5
		3	7.1	4.9	1.5	55	27	2.5	13	1	0.5	0.5
		5	6.9	7.05	0.5	44	36	2	15.5	1	1	1
		8	6.7	6.35	1	50	33	2	11	1	1	1.5
		12	6.4	6.6	3	52	30	1.5	11	1	1	2
	Post-dosing	17	6.7	7.25	0.5	46	33	2.5	13	1	1	2
		24	6.7	7.1	0	54	27	2	11.5	1.5	1.5	2
Sheep 22, l.w. 75 lb	Pre-dosing	1	6.3	8.7	0	58	25	2.5	12.5	1.5	1	1.5
		3	6.8	6.8	3.5	48	34	1.5	11	1	0.5	1
		5	6.7	6.75	0	38	41	4	14	1.5	1	1.5
		8	6.5	6.7	0	39	40	2.5	14.5	1.5	1.5	2
		12	6.5	7.0	0.5	49	32	2	13.5	0.5	1	1.5
	Post-dosing	17	6.5	6.9	0	48	33	2.5	13	1	1	2
		24	6.9	5.8	0	54	26	3.5	11	2	2	2

The sheep shown in this Table were members of the control and test groups of an experiment discussed in a previous paper (Jamieson 1958b). This work showed that there was an initial upset to the rumen fermentation during the second to sixth days of the nitrate regimen. The levels of total volatile fatty acids in the control sheep showed a slight fall over the experimental period. The dosed sheep (apart from the initial setback) did slightly better than the controls.

Sheep 18 and 14 illustrate the occasional preliminary increase in the percentage of acetic acid with the accompanying fall in the percentages of n-butyric and propionic acids. In these animals this is associated with a low value for the total volatile fatty acids as it was with sheep B in Table 1.

All dosed sheep in Table 3 showed the depression in the molecular percentage of acetic acid and differential rise in propionic and n-butyric acids after nitrate dosing. Sheep 22 shows the greatest change in that on two successive samplings levels of 37.5% and 39% were obtained, while the percentage of propionic acid was slightly greater than this. These values match the lowest molecular percentages of acetic acid obtained from normal sheep on ryegrass and clover during the survey summarised in Table 1.

In the present experiment there was no significant change in rumen liquor ammonia-nitrogen values. These fell and subsequently rose during the experimental period with the values for dosed and control animals showing virtually identical behaviour.

There were no increases in the molecular percentages of the branched-chain fatty acids of the rumen liquor such as accompanied the lowered acetic acid molecular percentages in the survey of normal animals. It thus appears that the metabolism of protein as measured by ammonia-nitrogen and branched-chain fatty acid concentrations was dependent on the pasture being consumed at the time of the experiment, and was not influenced by the nitrate dosing.

Sheep Dosed Nitrite

One sheep of 66.5 lb live weight was dosed 2 g of sodium nitrite daily for five successive days. Two other sheep 65–70 lb live weight, were given 2 g of sodium nitrite for three successive days. There was no marked change in the values for pH, ammonia-nitrogen or total volatile fatty acids, as between dosed and control sheep.

The depression of the molecular percentage of acetic acid produced by this treatment was not as severe as that obtained with potassium nitrate; possibly because of the relatively smaller amount of nitrite dosed. The results from only one animal have been quoted in Table 4. The other two sheep dosed showed a definite but smaller response in the lowering of the molecular percentage of acetic acid with a correspondingly slight rise in propionic and n-butyric acids.

The change obtained with nitrite appeared to be more transient than that with nitrate in that the values tended to return to their initial level during dosing. There was a considerable rise in the

percentage of propionic acid while that of n-butyric acid was barely detectable. There was no significant change in the proportions of the higher acids.

Sheep Dosed Hydroxylamine Hydrochloride

This compound is known to be highly toxic to almost all living organisms. The dose chosen was such as would not exceed that possible from the maximal conversion of 2 g of sodium nitrite in the rumen to free hydroxylamine.

Analyses on samples from two sheep during and after dosing with two grammes of hydroxylamine hydrochloride are given in Table 5. In sheep 21 the compound was administered in the form of free hydroxylamine by neutralising the hydrochloride with the calculated amount of sodium hydroxide just before dosing. The pH values remained within normal limits throughout the experiments. The values for total volatile fatty acids showed a marked fall during and after dosing. Smaller changes in ammonia-nitrogen occurred, and these paralleled the changes in total volatile fatty acids. These changes may be indicative of a loss of appetite on the part of the animals, or an inhibition of functional activity on the part of the rumen flora during this period; although no indication of the latter was given by an examination of the Gram stain and degree of fibre parasitisation of rumen contents, which showed a good to excellent grading over the whole experimental period. The total volatile fatty acid and ammonia-nitrogen values returned to normal in about one week after stopping the treatment.

It will be seen that there has been no significant change in the molecular percentage composition of the volatile fatty acids.

During these experiments it was found that the oral dosing of hydroxylamine caused a severe haemolytic anaemia, occasionally accompanied by haemoglobinuria in the treated sheep.

Sheep Dosed Hydrazine Sulphate

This compound is a theoretical reduction product of nitrite and nitrate. There is no evidence that it occurs in the rumen or is involved as an intermediate in the reduction of nitrate to ammonia. However, in view of the fact that hydrazine may be regarded as an analogue of ammonia it was considered of interest to test its effect on the rumen volatile fatty acid fermentation. The results obtained are given in Table 6, and the lower molecular percentages of acetic acid shown in the pre-dosing control samples of these sheep compared with those of Tables 2 -5 are due to changes in the pasture being grazed at the time of the experiment.

The rumen liquor pH values showed a slight elevation after dosing. There was no change in the E_h values, nor was there any marked change indicated by the values obtained for ammonia-nitrogen although here the method of determination may have been subject to interference from the dosed hydrazine.

TABLE 4. RUMEN V.F.A. OF SHEEP DOSED WITH 2 g SODIUM NITRITE DAILY FOR 5 DAYS
(First dose after sampling on 1st day; last dose on 5th day)

Animals and Treatment	Samples	Day	pH Rumen Contents	Total V.F.A. mM/100 ml	Individual V.F.A.				Molecular Percentages			
					Formic	Acetic	Pro-prionic	i-Butyric	n-Butyric	i-Valeric	α-Methyl-butyric	n-Valeric
Sheep 5; l.w. 66.5 lb	Pre-dosing	1	6.6	8.55	0	58	24	2.5	11.5	1.5	1.5	1.5
		2	—	6.9	2	41	36	2	15	1	1	2
		3	6.6	6.85	0	43	37	2.5	13	1.5	1.5	1.5
	Post-dosing	7	6.8	6.85	0	54	29	2	11	1.5	1.5	1.5
		10	—	5.8	0	59	25	2	10	1.5	1.5	1.5
		15	6.5	8.7	0	56	24	2	13.5	2	1	1.5
Mean of 2 controls		3	6.7	7.0	0	59	22	2.5	13	1.5	1	1
		8	6.8	6.2	0	56	23	2.5	14	2	1.5	1.5
		15	6.9	7.4	0	57	23	2.5	13	1.5	1	2

TABLE 5. RUMEN V.F.A. OF GRAZING SHEEP DURING ORAL DOSING OF HYDROXYLAMINE

Sheep 21, l.w. 68 lb	Pre-dosing	1	6.0	6.25	0	58	21	3	13	2	2	1.5
2 g hydroxylamine hydrochloride after sampling on 1st day		4	—	7.9	0	57	24	2.5	12	2	1.5	1.5
2 g neutralised hydroxylamine hydrochloride on 5th and 6th days		5	7.1	4.8	0	52	22	4	14	3.5	2.5	2.5
		6	7.4	3.2	0	55	21	4.5	11.5	4.5	2	2
	Post-dosing	7	7.1	4.2	0	58	21	4.5	9	3	3	2
		13	6.6	9.45	0	57	24	2.5	12.5	1	1.5	1.5
Sheep 20, l.w. 56 lb	Pre-dosing	1	—	9.75	0	55	25	2.5	13	1.5	1.5	1.5
2 g hydroxylamine hydrochloride after sampling on 1st day and again on 2nd day		2	6.8	6.5	0	57	22	2.5	13.5	2.5	1.5	1.5
	Post-dosing	3	6.8	4.45	0	59	20	3.5	12	2	2	1.5
		4	6.7	5.45	0	57	24	3	13	1.5	1	1
		10	6.7	8.25	0	59	22	2	13	2	1.5	1.5

TABLE 6. RUMEN V.F.A. OF SHEEP DOSED WITH HYDRAZINE SULPHATE
(2 g after samplings on 1st and 2nd days)

Sheep 5, l.w. 66lb	Pre-dosing	1	6.5	8.0	0	50	28	2.5	16	1.5	1	1
		2	7.2	5.0	0	50	25	2.5	17.5	2	1.5	2
	Post-dosing	3	8.0	2.75	0	43	30	3	19.5	2	2.5	0.5
		7	—	8.5	0	55	26	1.5	13.5	1	1	2
Sheep 8, l.w. 64 lb	Pre-dosing	1	6.5	8.8	0	50	28	3	15.5	2	1	1
		2	7.0	6.95	0	45	29	3	18	2	2	1
	Post-dosing	3	6.3	10.65	0	39	33	3	20	2	1.5	1

The total volatile fatty acids showed a marked depression in sheep 5 but not in sheep 8. The molecular percentages of the individual volatile fatty acids in both sheep showed changes quite comparable with those found after nitrate and nitrite dosing contrasted with the stability found after hydroxylamine administration.

The dose given proved toxic to both animals. On the second day both were in a very weak state, their saliva appeared to be dry, white, and frothy. The rumen contents were brown and drier than usual, and had a foul odour. Examination of the Gram stain showed a decline from a typical rumen flora with good fibre parasitisation to one showing that all organisms had apparently been adversely affected. One of the sheep killed on the second day for post-mortem examination showed mild universal liver damage and slight kidney damage.

The surviving animal recovered rapidly with the cessation of dosing. At no stage was haemoglobinuria or haemolytic anaemia observed.

Discussion

The results presented have shown, firstly, that changes occur in the molecular percentages of the rumen volatile fatty acids of sheep on pasture. This effect showed seasonal variations (unpublished results) and was presumably dependent on pasture composition. Secondly, it has been shown that similar changes in the molecular percentage composition of the principal acids, acetic, propionic, and n-butyric, may be induced by the administration of nitrate and certain of its reduction products.

Alterations in the molecular percentage composition of the volatile fatty acids have been reported to occur during the post-absorptive period for particular foodstuffs. Gray and Pilgrim (1951) found fairly extensive changes four to seven hours after feeding wheaten hay. The effect after feeding lucerne hay was much smaller, and similar to the changes found by Johns (1955b) for pasture-fed sheep and to the values for the control sheep reported here. Repeated samplings on the same day on free-grazing sheep showed only slight shifts in the relative concentrations of the various acids. Fasted sheep showed a profound fall in the level of total volatile fatty acids in the rumen liquor but no marked changes in the molecular percentages of the indivdual acids. Williams and Christian (1956b) obtained very little difference in sheep fed dried grass of uniform composition at various levels of intake, although these levels were strongly correlated with the prevailing total volatile fatty acid concentration.

The fact that the figures of Table 1 showed no significant correlation between the level of total volatile fatty acids and the molecular percentage of acetic acid indicates that the low concentrations of acetic acid that can occur are independent of the degree of rumen fill and are related to feed composition rather than feed intake.

Previously published analyses of the rumen volatile fatty acids from sheep on New Zealand pasture (Johns 1955b; Williams and Christian 1956a) showed molecular percentages of acetic acid all lying within the

range 53% to 67%, and did not exhibit the marked variations reported here.

Lowered acetic acid percentages comparable to the lower limit shown in Table 1 have been recorded overseas in samples from ruminants on various diets. Phillipson (1952) found lowered acetic acid levels, accompanied by an abnormally high propionic acid level in the volatile fatty acids present in the rumen of lambs fed a flaked maize ration. Sheep fed on high-protein diets containing ground-nut meal and maize showed a lowered molecular percentage of acetic acid and raised percentages of propionic and n-butyric acids together with increased proportions of the branched-chain acids (Annison 1954).

Balch and Rowland (1957) in a study of the rumen volatile fatty acids of dairy cows on a variety of diets including pasture, obtained a wide range in the molecular percentages of acetic acid values which varied inversely with the propionic acid. An increase in dietary protein resulted in increased percentages of butyric and the higher acids. These authors suggest that highly acid conditions may encourage the proliferation in the rumen of organisms that lower the proportion of acetic acid. It had been found that pasture feeding gave the lowest sustained pH values in the rumen. Only traces of lactic acid were formed after all diets except those with large amounts of flaked maize.

In an investigation of the production of volatile fatty acids from grass by rumen liquor in an artificial rumen, Barnett and Reid (1957) found that dried grass samples yielded a greater proportion of acetic acid than propionic compared with the corresponding fresh material and suggested that this is due to the increase in free sugars which occurs in dried grass. Aqueous extracts of grass contained the chief source of the acetic acid, while the fermentation of cellulose produced propionic acid in the greatest relative yield.

The results from the survey of normal animals shown in Table 1 differ markedly from those obtained in the various dosing experiments with reference to the ammonia-nitrogen, protein-nitrogen, and branched-chain acids. It has been shown by McDonald (1948) that there is a relationship between protein digestion in the rumen and the production of ammonia, and el-Shazly (1952b) found that rumen micro-organisms were able to deaminate amino-acids with the production of straight- and branched-chain volatile fatty acids and ammonia.

The figures for rumen liquor ammonia- and protein-nitrogen concentrations of Table 1 both show highly significant correlations with the concentration of the combined branched-chain acids, which would tend to link these three factors to the protein level prevailing in the pasture at the time of sampling. The fact that no significant correlation was obtained between ammonia-nitrogen or acetic acid concentrations and a negative correlation between the molecular percentage of acetic acid and ammonia-nitrogen of only a low significance (10% level) would tend to indicate that the effect involving the lowered molecular percentages of acetic acid and that due to increased protein metabolism in the rumen are largely independent of one another. As already mentioned, samples exhibiting lowered percentages of acetic acid tend to occur during spring and autumn—seasons during which

the crude-protein content of the herbage is liable to be abnormally high.

It will be seen from the results obtained during the dosing experiments that there was no change in the level of the branched-chain acids, while the ammonia values of the dosed animals behaved almost in identical fashion with those of the controls. It would thus appear that the metabolism of nitrate, or the other compounds dosed, had little or no effect on the protein metabolism of the pasture consumed during the experiments.

Certain similarities are apparent between the results from the survey of normal sheep and those dosed with nitrate, or nitrate-reduction products.

The significance of the increase in the proportion of formic acid which occurs in the normal animals of Table 1 is not clear. Gray *et al.* (1952) reported that formic acid was present in the rumen contents of sheep on wheaten or lucerne hay in levels ranging 0–5%, while Annison (1954) observed the infrequent occurrence of formic acid in small amounts, less than one per cent of the total volatile fatty acids. It has been found at Wallaceville that the occurrence of formic acid in the rumen of sheep on pasture is infrequent, but that there is a tendency for it to appear in sheep failing to grow well, and also in animals showing clinical signs of metabolic disorders of various kinds. The amounts involved were small, usually within the range 0–2% of the total volatile fatty acids; the highest value recorded being 4.0%. Formic acid appeared during the dosing of nitrate and nitrite in amounts up to 3.5% in the experiments of Tables 2, 3, and 4.

The range of molecular percentages of acetic acid found in the survey of normal sheep is the same as that which can be induced by nitrate dosing to an animal which on pasture alone would show an acetic acid percentage of about 60. In the dosing experiments during the period of lowest molecular percentage of acetic acid, the greatest compensatory rise has occurred in propionic acid with n-butyric acid showing only a slight increase. This may indicate, in view of Barnett and Reid's (1957) findings, that during these periods the volatile fatty acids produced in the rumen are those formed almost solely from the fermentation of cellulose.

The part played by the co-fermentation of carbohydrate in the efficiency of the reduction of nitrate in the rumen has been recognised. Sapiro *et al.* (1949) reported the marked protective action exerted by the administration of sucrose against methaemoglobinaemia caused by a dose of potassium nitrate. Lewis (1951b) showed that formate, succinate, lactate, citrate, glucose, malate, and mannitol, can act as hydrogen donors in the reduction of nitrate, but at lower rates than hydrogen. Holtenius (1957) found that carbon dioxide and total volatile fatty acid production was inhibited in the presence of nitrite and suggested that this might be due to the removal of keto-acids as oximes and possibly an interference with the Krebs cycle. The addition of glucose, fructose, lactate, or pyruvate, resulted in an accelerated removal of added nitrite from rumen contents. With Clark and Quin (1951), Holtenius found that while the reduction of nitrate to nitrite always

occurred, the further reduction of nitrite to ammonia was inhibited in sheep on a poor or inadequate diet and that this led to a comparatively greater degree of methaemoglobinaemia.

The relative constancy of the values for soluble sugar in New Zealand pasture and their low absolute values compared with those obtained elsewhere was noted by Johns (1955a). In an investigation of the effect of light and temperature on the chemical composition of pasture plants, Bathurst and Mitchell (1958) found that in general the soluble-sugar content changed inversely with change in the nitrate level in the plant. Values given by them for the non-volatile organic acids in the plant, although not extensive, appear to indicate that these also may vary inversely with the prevailing nitrate level.

It was found by Johns (1955a) that the crude-protein content of pasture showed relatively little decrease with the stage of growth, and Bathurst and Mitchell (1958) reported that the amount of insoluble nitrogen did not vary greatly under the different conditions of light and temperature, whereas the total nitrogen showed a much greater variation due to the changes in soluble nitrogen, which included nitrate-nitrogen.

As far as can be judged, the rumen flora is capable of handling the metabolism of nitrate with a reasonable degree of efficiency (Jamieson 1958b) and under normal conditions the sheep on nitrate-containing pasture is capable of carrying nitrate reduction through to ammonia which is a normal end-product of rumen fermentation.

It is concluded that the effects found on the molecular percentage composition of the volatile fatty acids in the rumen of sheep on pasture are due to major changes occurring in pasture composition. Nitrate, a known variable constituent of pasture plants, can during its fermentation in the rumen modify the composition of the volatile fatty acids. The rumen volatile fatty acids of low acetic and high propionic acid proportions found during nitrate and nitrite dosing may be the result of a ruminal fermentation lacking certain precursors of acetic acid normally present, with the results of Table 1 representing a similar fermentation complicated by the presence of greater amounts of protein.

Since nitrate metabolism in the rumen is known to require the presence of compounds which are capable of acting as hydrogen donors, and the most efficient of these are derived from the ruminal fermentation of monosaccharides, it is possible that any effect due to a high nitrate level in the pasture may be exacerbated by the concomitant low value for soluble sugar. The effects reported in the present work may thus be due to substrate competition involved in satisfying the requirements for nitrate reduction and acetic acid production.

The finding during the course of the present work that hydroxylamine, an intermediate in the reduction of nitrate to ammonia, can cause haemolytic anaemia in sheep has been further studied and a preliminary report on this aspect of the investigation given (Jamieson 1958a).

REFERENCES

Annison, E. F. 1954: *Biochem. J. 57:* 400-5.
Balch, D. A.; Rowland, S. J. 1957: *Brit. J. Nutr. 11:* 288-98.

Barnett, A. J. G.; Reid, R. L. 1957: *J. agric. Sci. 48:* 315-21; *49:* 171-83.
Bathurst, N. O.; Mitchell, K. J. 1958: *N.Z. J. agric. Res. 1:* 540-52.
Clark, R.; Quin, J. I. 1951: *Onderstêpoort J. vet. Sci. 25:* 93-103.
Gray, F. V.; Pilgrim, A. F. 1951: *J. exp. Biol. 28:* 83-90.
Gray, F. V.; Pilgrim, A. F.; Rodda, H. J.; Weller, R. A. 1952: *Ibid. 29:* 57-65.
Holtenius, P. 1957: *Acta agric. Scand. 7:* 113-63.
James, A. T.; Martin, A. J. P. 1952: *Biochem. J. 50:* 679-90.
Jamieson, N. D. 1958a: *Nature, Lond., 181:* 1601-2.
————1958b: *N.Z. J. agric. Res. 2:* 96-106.
Johns, A. T. 1955a: *N.Z. J. Sci. Tech. A37:* 301-11.
————1955b: *Ibid. A37:* 323-31.
Lewis, D. 1951a: *Biochem. J. 48:* 175-80.
——— ——1951b: *Ibid. 49:* 149-53.
McDonald, I. W. 1948: *Biochem. J. 42:* 584-7.
Phillipson, A. T. 1952: *Brit. J. Nutr. 6:* 190-8.
Sapiro, M. L.; Hoflund, S.; Clark, R.; Quin, J. I. 1949: *Onderstepoort J. vet. Sci. 22:* 357-72.
Shazly, K. el- 1952a: *Biochem. J. 51:* 640-47.
————1952b: *Ibid. 51:* 647-53.
Williams, V. J.; Christian, K. R. 1956a: *N.Z. J. Sci. Tech. A38:* 268-76.
——— ——1956b: *Ibid. A38:* 403-6.

ROOT ANATOMY OF GRASSES AND CLOVERS

Lolium perenne L., *Dactylis glomerata* L., *Glyceria fluitans* Br., *Alopecurus pratensis* L., *Trifolium repens* L., *T. pratense* L., and *Lotus uliginosus* Schkuhr (*major* Sm.)

KATHLEEN SOPER*, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 29 July 1958*)

Summary

Comparisons are made of the anatomy of the mature parts of roots of the grasses *Lolium perenne, Dactylis glomerata, Glyceria fluitans,* and *Alopecurus pratensis.*

Noteworthy features include a well-marked exodermis and a subjacent layer of sclerenchyma in *Glyceria;* the greater lignification of the *Dactylis* root as compared with *Lolium;* the greater total cross-sectional area of metaxylem vessels in *Dactylis* compared with the other three species; and the degree of development of lysigenous lacunae, which was least in *Dactylis* and greatest in *Glyceria* and *Alopecurus.*

Structural similarities are noted between the roots of *Glyceria* and *Oryza* (rice).

The structure of the cortex, particularly the development of lysigenous lacunae, is discussed in relation to habitat of the plant, aeration of the root, and absorption of water and nutrients.

The anatomy of mature parts of roots of the legumes *Trifolium repens, T. pratense,* and *Lotus uliginosus,* and of the mature *Lotus* nodule are briefly described.

T. repens and *T. pratense* had considerable secondary growth, a periderm developed in the pericycle, and the cortex and endodermis were sloughed off. *Lotus* had less secondary growth and retained its cortex for longer.

INTRODUCTION

Relatively little is known of the anatomy and histology of the roots of pasture plants and weeds. In 1934 Arber wrote "Our knowledge of the anatomy of grass roots is in a most unsatisfactory state. A great many individual observations have been made, but a section cut here and there is not enough and we still await a broad and thorough study of the structural relations, not only of roots of comparable ages and orders belonging to different species, but also of the different regions of the root . . .". From work on the decay of nodules and root tissue of white and red clover and *Lotus* as affected by defoliation and shading (Butler, Greenwood, and Soper, unpublished work), greater knowledge of the anatomy of the roots of these species also appears desirable.

This paper is presented not as a complete study of the root anatomy of any species, but as observations so far made on the root anatomy of various species. It is hoped that this information, although of restricted range, will be of use in present work and problems in the agricultural field, and will also open the way to further anatomical studies on these and other species.

* Mrs. C. S. W. Reid.

Some of the information presented here is available elsewhere, particularly much of that on white clover (Erith 1924). The inclusion of such material in this paper is justified by its use in comparisons with other species. While a number of workers have investigated the internal structure of nodules of various plants (e.g. Fred *et al.* 1932; Chen and Thornton 1940; Bottum 1941; Bond 1948; Arora 1956), as far as the writer is aware there is no record in the literature of the anatomy of the *Lotus* nodule. A brief description is therefore included here.

Methods

Plants of *Lolium perenne* (perennial ryegrass), *Dactylis glomerata* (cocksfoot), *Trifolium repens* (white clover), *T. pratense* (red clover), and *Lotus uliginosus* (*major*) were grown from seed, and plants of *Glyceria fluitans* (floating sweet-grass) and *Alopecurus pratensis* (meadow foxtail) from tillers obtained from their natural wet habitats. The plants were grown in a mixture of soil and sand, adequately fertilised and watered, in pots in a glasshouse from October until mid December 1957. Further plants of *Glyceria, Alopecurus,* and *Lotus* were grown until the following autumn, some of *Glyceria* and *Alopecurus* in water-logged soil.

Material for sectioning was obtained from the mature upper parts (excluding the top 1–2 inches) of nodal roots of the grasses; from branches of the taproot (secondary roots) of red clover; and from secondary roots and older adventitious roots of white clover and *Lotus.* The material was fixed in formalin acetic alcohol, a mixture of 5 parts formalin, 5 parts acetic acid and 90 parts 70% ethyl alcohol, and dehydrated and embedded in paraffin wax by the tertiary butyl alcohol method. Sections were cut 10μ thick and stained, usually in safranin and Delafield's haematoxylin. A combination of stains similar to that used for starch in nodules by Chen and Thornton (1940) was found very useful for legume roots. Sections were stained with haematoxylin for 2 min, left in 2% aqueous tannin for 12 h, stained in 1% aqueous gentian violet for 2 min and differentiated in 95% ethyl alcohol, then counter-stained with orange G for 5 min. This method gave better differentiation of fibres, cambium and starch.

The anatomical terms employed are those of Esau (1953), to which the reader is referred for further definition and usage.

Results

Comparison of Root Systems of Dactylis glomerata and Lolium perenne

Compared with *Lolium perenne,* there were fewer nodal roots, but almost twice as many roots per tiller, in *Dactylis glomerata* (Table 1). The extent of branching appeared similar in the two species, but no measurements were made. The cortex was intact and densely spaced root hairs were present throughout both root systems.

The root of *Dactylis* was wider but the ratio of stele to cortex was similar, the diameter of the stele being two-fifths of that of the whole root (Table 1). In neither species was an exodermis morphologi-

TABLE 1. NUMERICAL DATA FOR GRASSES
Mean values and in some cases standard errors given, *n* in brackets

	Lolium perenne	*Dactylis glomerata*	*Glyceria fluitans*	*Alopecurus pratensis*
Tiller number	88 ± 6 (9)	37 ± 3 (9)		
Nodal root number	103 ± 8 (9)	83 ± 4 (9)		
Number of roots per tiller	1.2	2.2		
Diameter of root (mm)	0.56 ± 0.03 (15)	0.75 ± 0.03 (15)	0.78 ± 0.04 (7)	0.69 ± 0.04 (7)
Width of cortex + epidermis (mm)	0.16 ± 0.01 (15)	0.21 ± 0.01 (15)	0.31 ± 0.02 (7)	0.25 ± 0.01 (7)
Diameter of stele (mm)	0.23 ± 0.01 (15)	0.32 ± 0.01 (15)	0.16 ± 0.01 (7)	0.19 ± 0.02 (7)
Stele/root	0.41	0.43	0.20	0.27
Number of protoxylem poles	10.9 ± 0.4 (15)	18.1 ± 0.8 (15)	7.1 ± 0.3 (7)	9.1 ± 0.6 (7)
Number of large metaxylem vessels	2.5 ± 0.3 (15)	7.2 ± 0.6 (15)	1.1 ± 0.2 (7)	1.4 ± 0.2 (7)
Diameter of large metaxylem vessels (μ)	49 ± 2 (39)	51 ± 1 (60)	66 ± 3 (7)	49 ± 4 (9)
Total cross-sectional area of large metaxylem vessels (mm^2)	0.0047	0.0147	0.0038	0.0026
Proportion of intercellular space in cortex (width of space/width of cortex)	0.5	0.3	0.6	0.4

cally differentiated from the underlying cortical parenchyma (Fig. 1A and B). The endodermis had its radial and inner tangential walls thickened (Fig. 2A and B). *Dactylis* had a lower proportion of lysigenous lacunae in the cortex than had *Lolium perenne* (Table 1). Sclerification of the cortical parenchyma occurred in both species. The sclerenchyma, when present formed a sheath, one, sometimes two, cells thick around the endodermis (Figs. 1 and 2). It occurred more frequently in *Dactylis* than in *Lolium*.

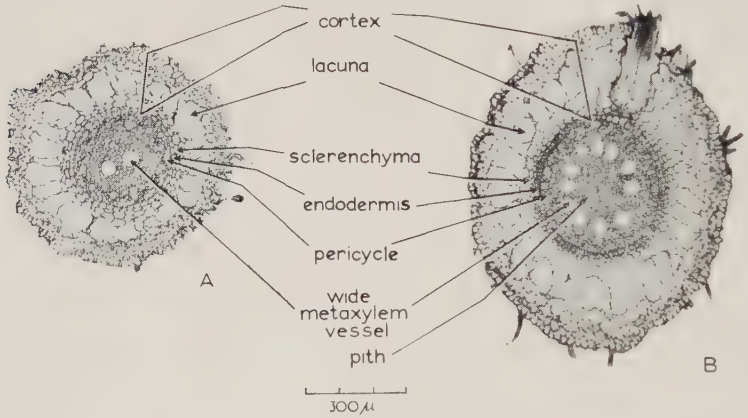


Fig. 1.—Transverse sections of roots. A, *Lolium perenne*. B. *Dactylis glomerata*. The base of a branch root is shown in the upper part of the section of *Dactylis*.

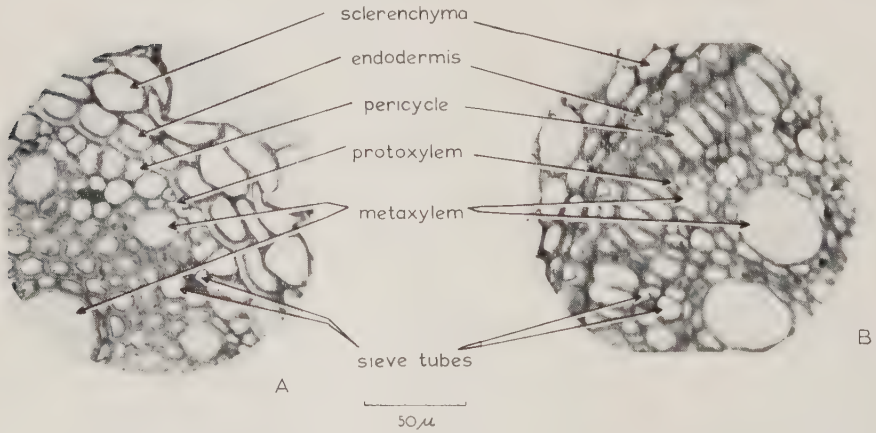


Fig. 2.—Tranverse sections of inner parts of roots shown in Fig. 1. A, *Lolium perenne*. B, *Dactylis glomerata*.

The pericycle in both species was uniseriate. It was usually sclerified, partly or entirely in *Lolium*, entirely in *Dactylis*. In *Lolium* but not in *Dactylis*, the pericycle was interrupted by the differentiation of xylem next to the endodermis (Fig. 2). There were more protoxy-

lem poles in *Dactylis* (Table 1). In both species the peripheral xylem strands were separated by non-tracheary elements from more interior, wide, metaxylem vessels. In *Dactylis* several large metaxylem vessels, circular to oval in transverse section, were arranged in a circle around a pith (Fig. 1B), whereas, in *Lolium,* 2 or 3 large vessels, circular in transverse section, occupied the centre of the vascular cylinder (Fig. 1A). The vessels were of similar diameter; but the total cross-sectional area of large vessels was in *Dactylis* three times as great as in *Lolium* (Table 1). In older roots of both species the parenchymatous cells separating the tracheary elements from each other and from the phloem strands were sclerified. The pith in *Dactylis* consisted of parenchyma similar to that located among the vascular elements, but sometimes with thinner walls. There was a tendency towards hollowness of pith in the wider roots.

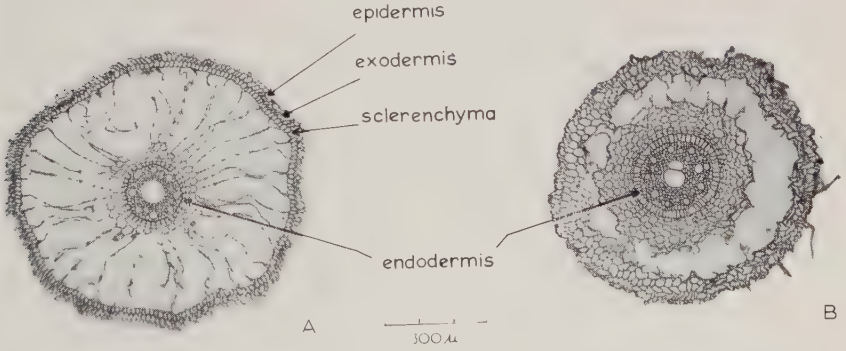


Fig. 3.—Tranverse sections of roots. A, *Glyceria fluitans.* B, a young root of *Alopecurus pratensis.* In older roots of *Alopecurus* all walls of the endodermal cells, including the outer tangential walls, were conspicuously thickened and lignified. In the section shown, the walls of the passage cells are not yet thickened (see also Fig. 4B).

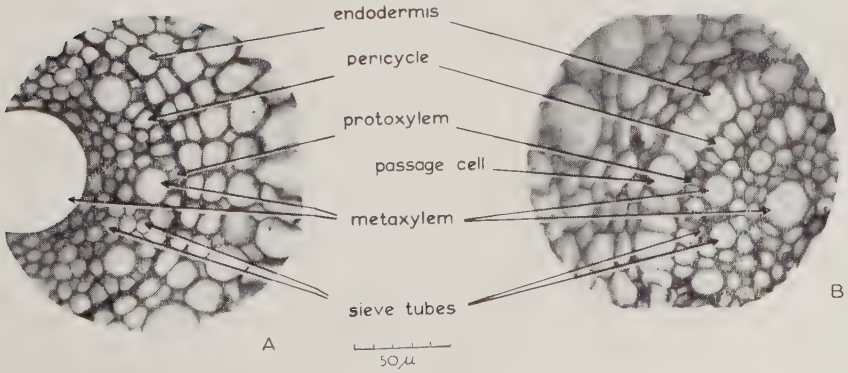


Fig. 4.—Transverse sections of inner parts of roots shown in Fig. 3. A, *Glyceria fluitans.* B, *Alopecurus pratensis.*

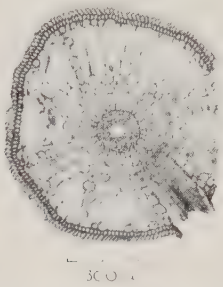


Fig. 5.—Tranverse section of root of *Glyceria fluitans*, from an autumn-harvested plant, illustrating the thick-walled sclerenchymatous layer beneath the exodermis, and the regular arrangement of cells and schizogenous intercellular spaces in the inner cortex (see also Fig. 6).

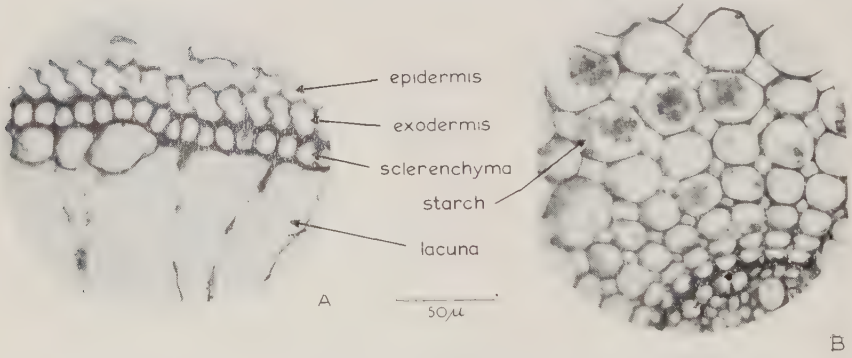


Fig. 6.—Tranverse sections of parts of the *Glyceria* root shown in Fig. 5. A, outer part. B, inner part, showing starch in the cells of the inner cortex.

Glyceria fluitans

Glyceria (Figs. 3A, 4A, 5, 6) had a thin-walled epidermis and a distinct exodermis. In some roots the epidermis, and with it the root hairs, had disappeared and was replaced by the exodermis as the outermost layer. Beneath the exodermis was a layer of sclerenchyma whose outer tangential cell walls were conspicuously thickened and lignified. Its radial and inner tangential cell walls also often became thickened and lignified, though usually to a lesser extent. The endodermis was similar to that of *Lolium* and *Dactylis*, with its radial and inner tangential walls thickened. The middle cortex was occupied by large lysigenous lacunae separated radially by narrow strands of cells or the remains of cell walls. The inner cortex consisted of cells which were commonly radially aligned, and of symmetrically arranged schizogenous intercellular spaces. The cortical layer next to the endodermis was sometimes slightly sclerified.

The diameter of the vascular cylinder was not more than one-fifth of that of the whole root (Table 1). The pericycle became slightly

sclerified in older roots. It was interrupted by small protoxylem elements. There were usually 7 protoxylem poles (Table 1), and almost invariably only one central metaxylem vessel which was separated from the peripheral xylem and phloem by parenchyma that became sclerified.

There were no consistent differences between roots obtained from swamps, roots grown in the glasshouse under the same conditions as *Lolium* and *Dactylis*, and roots grown in water-logged soil in pots in the glasshouse. Autumn-harvested roots, however, often had a lower proportion of lysigenous space in the cortex (Fig. 5), there being a wider inner cortex of cells which contained starch in abundance (Fig. 6B).

Alopecurus pratensis

Alopecurus (Figs. 3B, 4B), like *Lolium* and *Dactylis*, had no specialised exodermis. The most conspicuous layer was the endodermis which was unusual in that all its cell walls, including the outer tangential walls, were thickened. The cortex consisted of a few peripheral layers of cells separated from the inner layers by lysigenous spaces, with very few radial connections. The cells of the inner cortex were radially aligned. Sclerification of the cortical parenchyma occurred as in *Dactylis*, that is, in one or two layers of cells next to the endodermis in older roots.

The diameter of the vascular cylinder was approximately a quarter of that of the whole root (Table 1). The pericycle sometimes became lightly sclerified. It was interrupted by protoxylem elements. There were commonly about 9 or 10 protoxylem poles (Table 1) and 1 or 2 central metaxylem vessels which were separated from the peripheral strands by sclerified parenchyma.

Here again, there were no consistent differences between roots obtained from wettish pastures, roots grown under the same conditions in the glasshouse as *Lolium* and *Dactylis*, and roots grown in water-logged soils. Autumn-harvested roots appeared to have wider lysigenous spaces in the cortex than had those harvested in the early summer.

Trifolium repens, T. pratense, and Lotus uliginosus

All three species are herbaceous dicotyledons with secondary growth (Figs. 7, 8A). The internal organisation of tissues in white and red clover roots was similar, but red clover had produced more secondary tissue. Periderm had developed in the pericycle, and the cortex and endodermis were sloughed off.

Lotus had more limited secondary growth and the cortex persisted longer. The greater part of the cortex was occupied by large lysigenous lacunae. Darkly-staining masses of what was possibly tannin occurred in the inner cortex and often in the pericycle and secondary phloem. The endodermis was not clearly marked off as it was in the grasses.

Anatomy of Lotus Nodule

The nodule in *Lotus* is spherical. Its internal organisation is basically similar to that of other plants (e.g. Fred *et al.* 1932; Chen

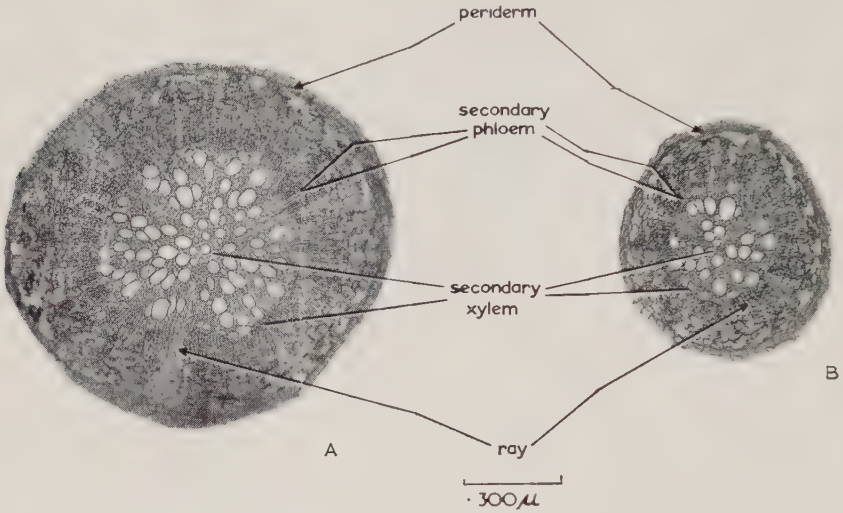


Fig. 7.—Tranverse sections of roots. A, *Trifolium pratense*. B, *T. repens*. *T. pratense* has produced more secondary tissue. In both, periderm has developed in the pericycle, and the cortex and endodermis have been sloughed off.

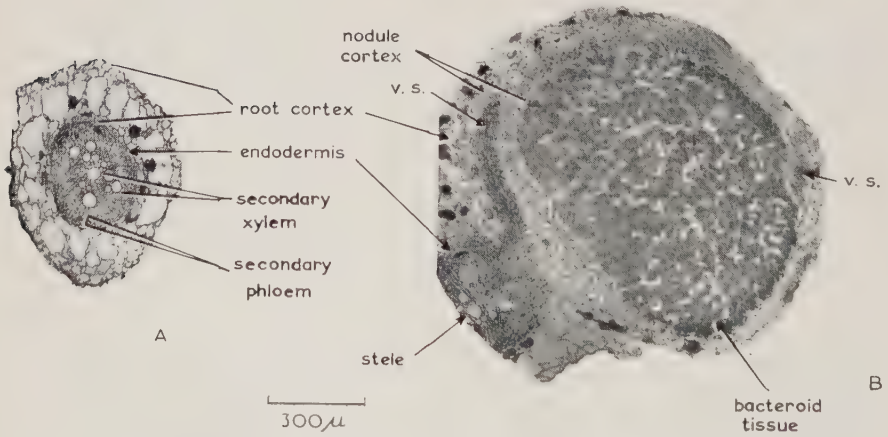


Fig. 8.—Tranverse sections of root and nodule of *Lotus uliginosus*. A, root. Secondary growth is small in amount and the cortex and endodermis have been retained. B, part of root with nodule. v.s. = vascular strand.

and Thornton 1940; Bottum 1941; Bond 1948; Arora 1956). The mature nodule shown in Fig. 8B consists mainly of bacteroid tissue surrounded by the nodule cortex, the whole embedded in the root cortex. Vascular strands in the nodule cortex connect with the stele of the root. Starch is present in abundance in the nodule cortex and also in the bacteroid tissue. The nodule meristem, which in the elongated nodules of white and red clovers is situated at the distal end of

the bacteroid region, is in *Lotus* not clearly defined and appears to lie on the outer periphery of the bacteroid zone.

DISCUSSION

Apart from the differences in morphology and in anatomy between the roots of monocotyledons and dicotyledons, the roots of the species investigated here exhibited marked internal differences which were associated with physiological specialisations or adaptations to environment.

The Grasses

Many roots develop a specialised layer—the exodermis—beneath the epidermis. No such layer was apparent in *Dactylis glomerata, Lolium perenne,* or *Alopecurus pratensis,* but *Glyceria fluitans* had a distinct exodermis which often replaced the epidermis as the outermost protective layer. Its cell walls never became very thick.

Sclerification of cortical cells is often found in the monocotyledons (Esau 1953, p. 477), and occurred in all the four species studied here. The sclerenchyma, when present, takes the form of a layer one or more cells in depth, either beneath the epidermis directly, or beneath an exodermis, or next to the endodermis. A sheath one or two cells thick next to the endodermis was much more common in *Dactylis* and *Alopecurus* than in *Lolium* or *Glyceria.* Sclerenchyma in the outer cortex occurred in *Glyceria* only, where it took the form of a layer one cell thick beneath the exodermis. The outer tangential walls of the sclerenchymatous cells were always conspicuously thick and lignified. The radial and inner tangential walls also were sometimes thickened and lignified, though usually to a lesser extent. The exodermis and accompanying subjacent layer of sclerenchyma strengthen the root and are probably important in preventing the collapse of the large lacunae in the cortex when the root is submerged in water. It is of interest that the outer layers of roots of *Oryza* (rice) (Erygin and Ryabchun 1957) show similar development.

The pericycle in the monocotyledons often undergoes sclerification in older roots (Esau 1953, p. 484). The parenchymatous cells separating the tracheary elements from each other and from the phloem strands also often become sclerified (Esau 1953, p.488). Both occurred in all of these species, sclerification of the pericycle to the greatest extent in *Dactylis.*

The roots of *Dactylis* thus had more lignified tissue than *Lolium* roots of comparable age. It has been suggested (Davies 1951) that the stronger root system of cocksfoot, as compared with ryegrass, may improve the texture of ploughed-up soil. This may be due to the greater lignification of the cocksfoot root.

As with the presence or absence of pith, the number of protoxylem poles is related to the diameter of the vascular cylinder. If the diameter is large the number of poles is greater and pith is more likely to be present than in roots with narrow vascular cores (Esau 1953, p. 485). This is well illustrated by *D. glomerata* and *L. perenne. Dactylis*

with the wider root had the larger number of protoxylem poles and a pith. Within each species also, the wider vascular cylinders generally had the greater number of protoxylem poles. In *Dactylis,* narrower roots had a pith whose cells were sclerified to a similar extent to those located among the vascular elements, but in larger roots the pith parenchyma had thinner walls and there was a tendency towards breakdown of the central cells to form a hollow pith.

In roots of both species, the diameters of the large metaxylem vessels were similar, but *D. glomerata* had a larger number of these vessels, and their total cross-sectional area was three times as great as in *L. perenne.* Emerson (1954), in his work on water conduction through severed grass roots, found a correlation between diameter of the conducting elements and rate of flow. It would be expected, therefore, that the flow through roots of *D. glomerata* would be some three times that through roots of *L. perenne.* Emerson found in his material, however, that the flow along roots of *D. glomerata* was actually smaller than that through roots of *L. perenne* because, although *Dactylis* had a large number of elements, in contrast to the findings in this work, the elements were smaller in diameter.

The structural characteristics in aquatic plants are mainly those of reduction of the protecting, supporting, and conducting tissues and the presence of air chambers (Eames and MacDaniels 1947, p. 388). Of the four species studied, *Glyceria* had least sclerenchymatous tissue in the vascular cylinder and inner cortex of the roots, the smallest vascular cylinder relative to the size of the whole root, and fewest xylem elements. The presence and significance of an exodermis and a subjacent layer of sclerenchyma have already been noted.

Alopecurus is not an aquatic but is adapted to growing in wet grasslands. The development of sclerenchymatous tissue in and around the vascular cylinder was more nearly comparable to that in *Dactylis* and *Lolium* than in *Glyceria.* The diameter of the vascular cylinder relative to that of the whole root, although somewhat larger than in *Glyceria,* was smaller than in *Lolium* or *Dactylis* and the number of xylem elements was intermediate between those of *Glyceria* and *Lolium* (Table 1). There was no development of specialised layers in the outer cortex like the exodermis and subjacent sclerenchyma in *Glyceria.*

Regularly arranged, large lacunae in the leaves and stems of aquatic plants are supposed to provide for the storage of, and act as reservoirs for gases, and to give buoyancy (Eames and MacDaniels 1957, p. 390). Cavities similar to the lacunae in the roots of *Glyceria* are present in the roots of *Oryza* (Erygin and Ryabchun 1957). They are described as "aerial" and as a source of oxygen, which accumulates there and may be secreted. The leaves of *Glyceria* float on water and have abundant stomata. Lacunae are present in the leaves and stems as well as in the roots. It appears likely, therefore, that air taken into the chambers of the leaves can spread throughout the plant.

The development of lysigenous spaces seems to have been carried to the furtherest extent in *Alopecurus* where in some cases there was almost continuous space between the outer and inner cortex.

Popham (1955), working with *Pisum* roots, found that continuous ducts, formed by superposed intercellular spaces in the cortex, extended from the level of the top of the columella throughout the length of the root. He suggested that these were possible avenues of rapid movement of water and solutes to meristematic cells lying nearer the apex than levels at which xylem and phloem are believed to become "functional".

Lundegårdh's (1950) experiments with wheat roots, however, support the conclusions that, while limited longitudinal translocation of salts occurs in the cortex of the tip zone, it is by way of *cells,* not spaces and that the intercellular spaces in the cortex of the root normally contain air (except during periods of extreme water requirements).

Briggs (1957) writes that for many solutes there is evidence that the free space (that is, that part of plant tissues into and through which solutes pass relatively freely) includes the cell walls, the cytoplasm, and the intercellular spaces which, because of the different solubility of oxygen and carbon dioxide, will become injected if the tissue is kept immersed in a solution.

Crafts and Broyer (1938) and Lundegärdh (1950) stressed the importance in the translocation of salts from epidermis to vessels of the gradient from the well aerated cortex to the poorly oxygenated stele. The possession of large lysigenous lacunae in *Glyceria* and *Alopecurus* can probably be regarded as of value in maintaining maximum oxygenation of the cortex under conditions of water-logging. Some experiments suggest that the development of large lysigenous intercellular spaces may be causally related to scarcity of oxygen (Bryant 1934, McPherson 1939). That it is an inherited character in *Glyceria* and *Alopecurus* rather than one due to the immediate effect of the environment is shown by a comparison with *Lolium* and particularly with *Dactylis* which had comparatively few and small lacunae, and by the similarities among plants grown in soils of different degrees of wetness. Erygin and Ryabchun (1957) found that rice roots grown under "flooded" as compared with "watered" conditions were larger, but there were no differences in the development of the aerenchymatous tissue which arises through the breakdown of cortical cells. These facts support the hypothesis of Peterson *et al* (1957) that a genotype of moister modal habitat has inherently wetter, more dilate tissues (greater fresh weight/dry weight ratio) than a similar related genotype of drier modal habitat.

Using the apical 7–10 cm of roots, Lundegårdh (1950) found that translocation of salts in the longitudinal direction through the cortex is of no practical importance (except in the tip zone) because the salts at all levels are transported primarily in the direction of the radius straight into the stele. It seems unlikely that this could be the case in older parts of roots where the endodermis, including the passage cells, has thickened cell walls and presumably functions as a barrier to transport from cortex to stele at higher levels in the root. Wiebe and Kramer (1954) concluded from their own and other experiments that the thickened endodermis presents no barrier to the inward movement of water and solutes. They do not appear, however, to have considered the possibility of downward movement of these materials through the

cortex to regions where the endodermis, or at least the passage cells, are not thickened, although their results showed that all of the isotopes used moved downward in the roots and that killing a narrow segment of root tissue with steam reduced the downward movement of ^{32}P.

The three species *Lolium, Dactylis,* and *Alopecurus* still had root hairs throughout their root systems when harvested, though whether or not these hairs could still absorb water and nutrients is not known. Should the root hairs and epidermis in the upper regions of roots still absorb, even to a limited extent, and if the endodermis does function as a barrier to radial transport, then longitudinal transportation through the cortex in the direction of the root tip may be important. This presumably could not be the case in older roots of *Glyceria* where the exodermis and subjacent sclerenchyma form a barrier to absorption. In the case of the rice root, Erygin and Ryabchun (1957) describe the woody sclerenchymatous sheath as "having isolated the root from the surrounding medium" and state that the central cylinder in the older parts of the root can establish connections with the soil only by means of lateral rootlets.

The Legumes

Some herbaceous dicotyledons lack secondary growth, others have mere vestiges of such growth and still others produce considerable amounts of secondary tissue (Esau 1953, p.491). In many of those having secondary growth, a periderm develops in the pericycle, and the cortex together with the endodermis, is ruptured and sloughed off. This occurred early in white and red clovers. In other species where the secondary growth is small in amount, the cortex is retained in the mature root (Esau 1953, p. 495). In *Lotus* the cortex was found to have been discarded only in the oldest roots of autumn-harvested plants.

Although *Lotus* resembles the grasses in the retention of its cortex there was no sclerification of the cortical cells, as is often found in the monocotyledons.

The cortex is the principal storage region for starch in monocotyledonous roots. In dicotyledonous roots, starch is accumulated in various parenchymatous and some sclerenchymatous cells of the secondary tissues (Esau 1953, p. 507). In the three legumes investigated here, starch was present in cells of the vascular rays and xylem and phloem parenchyma. *Lotus* was distinctive in the possession of darkly staining masses, possibly tannin, which were always present in the cells of the inner cortex, and sometimes in the pericycle and secondary phloem. Tannins are a group of polyphenol derivatives which, when the tissue is dehydrated, appear as granular masses, or bodies of various sizes, which are very conspicuous in sectioned material. They are often present in dicotyledons but seldom in monocotyledons.

Many primary features of the root seem to be associated with absorption. Numerous investigations show that during the period of active absorption by the plant, new primary areas bearing functional root hairs are constantly added (Kramer 1945). Furthermore, the extensive branching characteristic of most roots is responsible for the presence of numerous apical meristems that produce new primary root tissues and

extend existing ones (Esau 1953, p. 507). Some experimental evidence, however, suggests that, to a limited extent, absorption may occur in older root parts where secondary vascular tissues are present and the surface is covered by periderm (Kramer 1949).

ACKNOWLEDGEMENTS

The writer is indebted to the late Mr. E. O. C. Hyde and to Mr. C. S. W. Reid both of the Department of Scientific and Industrial Research, for suggestions and discussions on various aspects of this work, and to Mr. H. Drake, photographer, DSIR, for assistance with the figures.

REFERENCES

Arber, Agnes 1934: "The Gramineae. A Study of Cereal, Bamboo and Grass". Cambridge University Press, Cambridge.

Arora, N. 1956: Histology of the Root Nodules on *Cicer arietinum* L. *Phytomorphology 6:* 367-77.

Bond, L. 1948: Origin and Developmental Morphology of Root Nodules of *Pisum sativum*. *Bot Gaz. 109:* 411-34.

Bottum, F. R. 1941: Histological Studies on the Root of *Melilotus alba*. *Bot. Gaz. 103:* 132-45.

Briggs, G. E. 1957: Some Aspects of Free Space in Plant Tissues. *New Phytol. 56:* 305-24.

Bryant, A. E. 1934: Comparison of Anatomical and Histological Differences between Roots of Barley Grown in Aerated and Non-aerated Culture Solutions. *Plant Physiol. 9:* 389-91.

Chen, H. K.; Thornton, H. G. 1940: Structure of "Ineffective" Nodules and Nitrogen Fixation. *Proc. roy. Soc. B 129:* 208-29.

Crafts, A. S.; Broyer, T. C. 1938: Migration of Salts and Water into Xylem of the Roots of Higher Plants. *Amer. J. Bot. 25:* 529-35.

Davies, W. 1951: Plant and Animal Nutrition in Relation to Soil and Climatic Factors. *Proc. Specialist Conf. Agric. Brit. Commonwealth Sci. Off. Conf.:* 403-9.

Eames, A. J.; MacDaniels, L. H. 1947: "An Introduction to Plant Anatomy". McGraw-Hill, New York and London.

Emerson, W. W. 1954: Water Conduction by Severed Grass Roots. *J. agric. Sci. 45:* 241-5.

Erith, Adela G. 1924: "White Clover (*Trifolium repens* L.)". A Monograph. Duckworth, London.

Erygin, P. S.; Ryabchun, O. N. 1957: The Relation of the Structure of the Rice Root to the Water Regime of the Soil. *Doklady Akad. Nauk. SSSR 115:* 1028-30.

Esau, Katherine 1953: "Plant Anatomy". Wiley, New York.

Fred, E. B.; Baldwin, I. L.; McCoy, Elizabeth 1932: "Root Nodule Bacteria and Leguminous Plants." *University of Wisconsin Studies in Science, No. 5.* Madison.

Kramer, P. J. 1945: Absorption of Water by Plants. *Bot. Rev. 11:* 310-55.

————————1949: "Plant and Soil Water Relationships". McGraw-Hill, New York.

Lundegårdh, H. 1950: The Translocation of Salts and Water Through Wheat Roots. *Physiol. Plant. 3:* 103-51.

McPherson, D. C. 1939: Cortical Air Spaces in the Root of *Zea mays L. New Phytol. 38:* 190-202.

Petersson, M. L. R.; Ellis, S. K. F.; Gray, G. E. J.; Smith, R. S. 1957: An Inherent Character of Plant Species of Drier Habitats. *Nature 180:* 698-9.

Popham, R. A. 1955: Levels of Tissue Differentiation in Primary Roots of *Pisum sativum*. *Amer. J. Bot. 42:* 529-40.

Wiebe, H. H.; Kramer, P. J. 1954: Translocation of Radio-active Isotopes from Various Regions of Roots of Barley Seedlings. *Plant Physiol. 29:* 342-8.

BLOAT IN CATTLE

XIII. THE EFFECT OF ORALLY ADMINISTERED PENICILLIN ON THE FERMENTATION AND FOAMING PROPERTIES OF RUMEN CONTENTS

By J. L. Mangan, A. T. Johns, and R. W. Bailey, Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North

(Received for publciation, 8 October 1958)

Summary

Procaine penicillin (100,000–500,000 units) has been administered orally to one of a pair of fistulated identical-twin cows fed on cut red clover. A marked effect was produced on the foaming properties of the rumen contents of the treated animal, together with an inhibition of volatile fatty acid and ammonia production and of protein breakdown. Reducing sugars and lactic acid accumulated while soluble protein concentration rose rapidly during the feeding period.

The action of penicillin in preventing bloat appears to be due to its effect on gas production and on the foaming properties of the rumen contents. It is postulated that the chloroplast fat is acting as the anti-foaming agent in the treated animal. The effect of penicillin on the rumen fermentation disappeared on repeated dosing.

Introduction

As cellulolytic digestion within the rumen is due to the action of micro-organisms, the introduction of bacteriocidal or bacteriostatic agents into that organ does not seem logical. However, the finding of Barrentine *et al.* (1956), that 50 to 75 mg of procaine penicillin administered orally will protect steers from bloating for periods of up to three days, has made it desirable to study the effect of this antibiotic on the rumen fermentation.

Bloat is caused by the inability of the animal to eliminate the gas normally produced by the anaerobic fermentation of food in the first two stomachs. In the majority of cases of pasture bloat this is considered to be the result of gas being trapped in the foaming ingesta (Johns 1958).

It is believed that for bloat to occur the following events must take place in the rumen (Johns, Mangan, and Reid 1957):

(a) A vigorous fermentation to produce gas and fatty acids causing a lowering of the pH with the consequent additional gas production from the bicarbonate from the saliva.

(b) A concurrent release from the plant of foaming constituents (proteins and saponins), together with anti-foaming agents (fats).

Other foaming compounds which could contribute to foam formation are the mucoproteins of saliva and the slime-producing bac-

teria as suggested by Hungate *et al.* (1955). In an earlier paper in this series (Mangan 1959), it was found that the foaming properties of clover cytoplasmic protein approximate most closely those of rumen fluid. Both showed maximum foam strengths at pH values ranging from 5.4 to 6.0.

Penicillin could be acting by inhibiting specific organisms such as the slime producers, or by reducing the general level of fermentation and hence lowering the rate of volatile fatty acid (higher pH) and gas production.

Studies to date on the effect of penicillin on rumen organisms have been mainly confined to the use of *in vitro* techniques. For instance, Wasserman *et al.* (1952) found that 5 to 7.5 units of penicillin per ml stimulated cellulose digestion but that 15 units/ml were inhibitory. Hardie *et al.* (1953) and Huhtanen *et al.* (1954) on the other hand concluded that penicillin was inhibitory to cellulose digestion at all levels tried.

Andrews *et al.* (1957a, b) showed that while 50,000 units of penicillin fed daily to lambs was associated with moderate alteration of rumen function and increased growth rate, 150,000 units fed three times weekly was accompanied by marked and undesirable changes in rumen function and a decline in mean body weight.

The present paper presents results of an investigation of the effect of orally administered penicillin (100,000 to 500,000 units) on the fermentation in the rumen of cows and on the foaming properties of the rumen fluid.

METHODS

Animals

A pair of fistulated identical-twin cows were fed 35 lb (fresh wt.) of cut red clover over a period of 2 h, commencing at 8 a.m. Bloat grading was that used by Johns (1954).

Antibiotic

The antibiotic in the form of lactose-based tablets* of 100,000 units of procaine penicillin was administered through the fistula at 4 p.m. on the day prior to the experimental period.

Rumen Samples

Samples were withdrawn from within the rumen near the fistula, squeezed through muslin and rapidly cooled with ice to arrest fermentation. This fluid is termed crude rumen liquor. Clear rumen liquor, free from bacteria, was obtained by centrifuging for 30 min at 10,000 g in a Spinco preparative ultra-centrifuge using a pre-cooled rotor.

Measurement of Foaming Properties

Foam expansion and foam strength measurements were made on both crude and clear rumen liquors by the methods previously described (Mangan 1958).

* Tablets for the trials were kindly supplied by Glaxo Laboratories (N.Z.) Ltd., Palmerston North.

pH Measurements, using a battery-operated pH meter with a glass electrode, were made on the crude rumen liquor as soon as possible after removal from the animal.

The following determinations were carried out on the clear rumen liquor:

Volatile fatty acids were determined on 5 ml of liquid by the addition of 1 ml 10 N H_2SO_4 and 4 g $MgSO_4.7H_2O$ followed by steam distillation in the Markham Still. Quantitative recovery was obtained by collecting 50 ml of distillate which was titrated with 0.1 N NaOH under CO_2-free conditions.

Ammonia was determined by the method of Conway (1947) as applied to rumen contents by McDonald (1948).

Lactic acid was estimated by the method of Friedemann and Graeser (1933).

Penicillin was estimated in the rumen fluid by the method of Whitehead and Cox (1958). Samples of clear rumen fluid were deep frozen immediately after centrifuging and so kept until the dilutions in sterile milk were carried out.

Total soluble N was estimated by the method of Chibnall *et al.* (1943).

Soluble protein N. Sodium sulphate (6 g) was dissolved in a 20 ml aliquot of the liquor and the pH adjusted to approximately 5.5 with a few drops of glacial acetic acid. The precipitated protein was spun down by centrifuging at 13,000 g for 30 min. The protein was digested with 20 ml concentrated sulphuric acid and copper-selenium catalyst until clear. The mixture was cooled, diluted with water to 50 ml and total N estimated on 5 ml aliquots by the semi-micro method of Chibnall *et al.* (*loc. cit.*).

Amino N. The supernatant from the salt precipitation of soluble protein was made up to 50 ml with water. Aliquots (10 ml) were adjusted to pH 9 with sodium hydroxide and borate buffer. Free ammonia was removed by distillation for 10 min in vacuo at 40°C. The solution was neutralised with acetic acid, made to 25 ml with water, and 5 ml aliquots used for the estimation of amino N by the manometric method of Van Slyke (1929).

Reducing Substances

Clear rumen liquor (5 ml) was deproteinised by the method of Doak (1939) and the filtrate diluted to volume (25 ml). Reducing substances were measured in a portion of the solution (5 ml) by the cuprimetric method of Shaffer and Hartmann (1921). In the case of solutions containing low levels of reducing substances, the sensitivity of the method was increased by adding glucose solution (1 ml containing 1.00 mg) to the copper mixture before carrying out the reduction. A blank containing the added glucose plus water and copper reagent was also included and the concentration of reducing substances obtained by difference. Results were calculated as glucose and reported as mg of

glucose/100 ml of cell-free rumen liquor. All analyses were done immediately after centrifuging the cooled samples of rumen liquor.

Identification of Reducing Substances in Clear Rumen Liquor

Boiling ethanol (2 vol) was added to 200 ml of clear rumen liquor. The solutions were cooled to 20°c and centrifuged to remove the bulky protein precipitate. After concentration, under vacuum, the solution was desalted with Dowex 50 and Dowex 2 ion-exchange resins and evaporated to dryness in a vacuum desiccator. The residue was dissolved in water (1 ml) and analysed for sugars by paper chromatography. Papers were developed with a solvent consisting of the top layer of a mixture of ethyl acetate, water, and pyridine (2:2:1 by vol.; Jermyn and Isherwood 1949). Sugars were detected with silver nitrate (Trevelyan *et al.* 1950) and with aniline hydrogen phthalate (Partridge 1949).

RESULTS

Preliminary Trial

An experiment was carried out in which a pair of identical-twin animals were each fed 35 lb (fresh weight) of clover morning and

TABLE 1. SIMILARITY OF IDENTICAL-TWIN COWS IN THE PRODUCTION OF SOME RUMEN FERMENTATION PRODUCTS WHEN FED THE SAME AMOUNT OF RED CLOVER (35 lb)

Day	Sample Time After Feeding (h)	Cow	V.F.A.[1] (mM/100 ml)	T.S.N.[2] (mg N/100 ml)	Ammonia N (mg N/100 ml)	Intake Fresh (lb)	Bloat Score
1	0	293	7.6	18.4	9.6	0	0
	1		13.2	42.0	19.7	18.0	0
	2		16.3	44.2	23.4	33.9	0
	0	294	8.9	21.2	8.74	0	0
	1		15.1	43.4	21.9	17.6	0
	2		17.5	39.2	27.0	32.6	III
2	0	293	6.45	25.4	10.2	0	0
	1		12.30	51.0	19.3	18.2	I
	2		17.50	45.2	20.6	32.2	II
	0	294	6.24	21.4	8.5	0	0
	1		12.0	42.4	18.9	19.3	I
	2		15.7	51.6	20.8	34.5	II
3	0	293	7.56	14.4	7.9	0	0
	1		13.10	31.6	—	26.0	II
	2		16.10	38.2	23.2	25.9	III
	0	294	6.5	11.4	7.6	0	0
	1		11.9	30.4	19.0	29.3	II
	2		15.3	40.2	—	30.2	III-IV

[1] Volatile fatty acids
[2] Total soluble nitrogen

afternoon. This was to determine how similar the animals were in their production of fermentation products in the rumen when both were untreated. The fresh-weight intake was measured, and samples of rumen liquor taken at hourly intervals for chemical analysis. The results for the a.m. feedings are shown in Table 1. The p.m. feedings gave similar patterns for each member of the twin set.

It will be seen that the intake of herbage, the volatile fatty acid, the total soluble nitrogen and ammonia levels followed very similar trends in both animals. The bloating pattern was almost identical for the two animals for two out of the three days.

It was concluded that the use of a pair of identical-twin animals, fed the same amount of herbage, would be sufficient to show up a marked fermentation difference due to treatment of one of the pair.

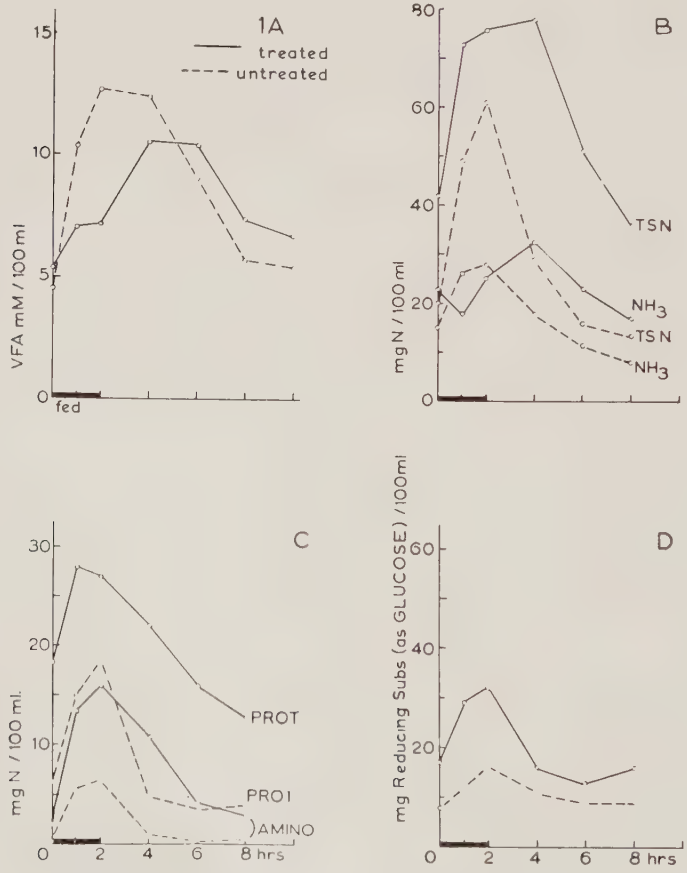


Fig. 1.—The effect of 100,000 units of procaine penicillin given orally 16 h previously on, A. volatile fatty acid (V.F.A.) level; B. total soluble nitrogen (T.S.N.) and ammonia (NH_3); C. proteins (Prot.) and amino acids; D. reducing substances concentration in the rumen.

Penicillin Treatment Trials

One of a pair of twin animals was treated with varying doses of penicillin at irregular intervals over a period of several months. The results of the first dosing with 100,000 units on the rumen fermentation pattern is illustrated in Fig. 1.

A marked difference in colour was apparent between the rumen contents of the treated and untreated animals. The former appeared

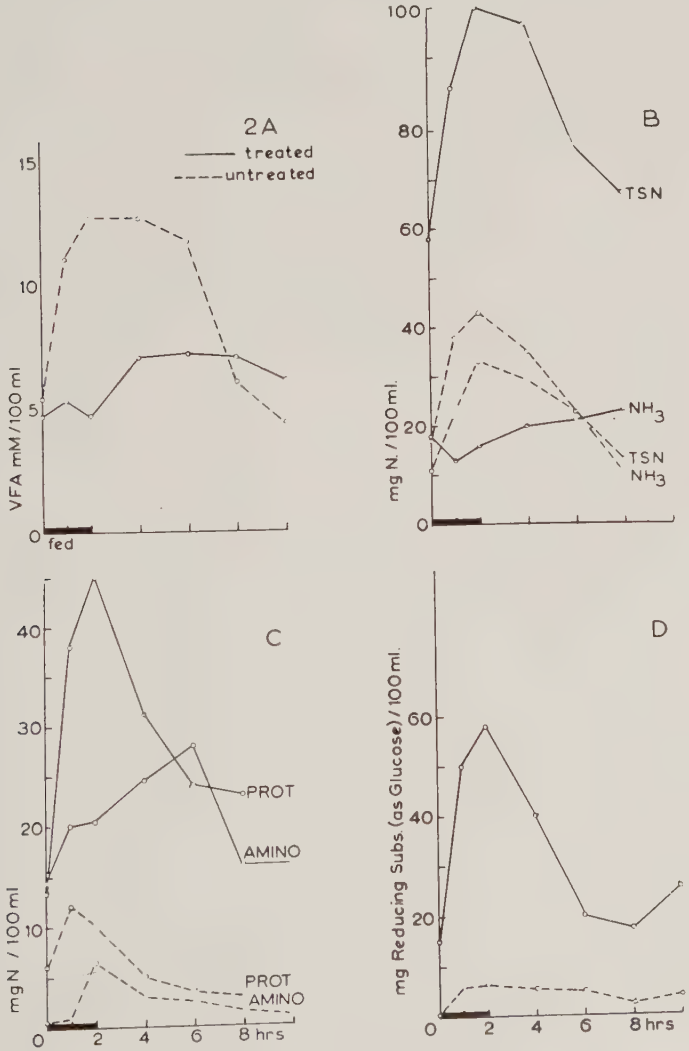


Fig. 2.—The effect of 500,000 units of procaine penicillin on rumen fermentation. A. volatile fatty acids; B. total soluble nitrogen and ammonia; C. proteins and amino acids; D. reducing sugar levels.

distinctly brown in contrast to the latter which showed the normal bright green of the rumen contents of clover-fed animals.

Some two weeks later the same animal was dosed with 500,000 units of procaine penicillin to obtain a more marked effect on the rumen fermentation. The results are shown in Fig. 2.

The treated animal showed no signs of bloat whereas the rumen contents of the control twin were markedly foamy and this animal was a Grade II bloat at 2 h after the commencement of feeding, despite the fact that the rumen fistula was being opened at intervals for the taking of samples.

The foaming properties of the crude and clear rumen liquor were investigated and the results, together with pH measurements, bloat grade, and nitrogen analyses, are recorded in Table 2.

TABLE 2. EFFECT OF 500,000 UNITS OF PENICILLIN ON THE FOAMING PROPERTIES OF RUMEN LIQUID

	Time from Feeding	pH	Bloat Score	Foaming Properties				Nitrogen Analysis (mg N/100 ml)	
				Crude		Centrifuged			
				F.E.[1] (cm)	F.S.[2]	F.E.[1] (cm)	F.S.[2]	Total Sol. N.	Sol. Protein
	(Hours)								
Control	0	6.82	0	8	nil	9	nil	16.6	6.5
	1	6.39	I-II	28	0.1	45	0.43	38.4	12.6
	2	6.19	II	45	0.64	45	0.31	43.4	10.0
	4	6.32	0	45	0.17	40	0.05	35.0	5.0
	6	6.38	0	45	0.18	6	nil	25.6	3.7
	8		0			11	nil	13.6	3.7
	10	6.80	0	45	0.16	36	nil	18.0	4.4
Treated	0	6.82	0	2	nil	9	nil	58.8	13.4
	1	6.41	0	6	nil	45	4.95	89.2	38.2 33.7
	2	6.30	0	4	nil	45	36.6	102.6	45.2
	4	6.29	0	2	nil	45	34.0	97.0	31.8
	6	6.39	0	4	nil	45	2.0	77.0	23.8
	8	6.45	0	—	nil	45	0.25	66.4	23.2
	10	6.61	0	5	nil	45	0.66	57.4	18.7

[1] Foam expansion
[2] Foam strength

After a lapse of one month, the same animal was dosed with 200,000 units of procaine penicillin on three occasions within a period of two weeks. The marked difference in fermentation pattern between treated and untreated animals narrowed until on the third dose there was little difference between the twin mates (see Fig. 3).

Unfortunately no bloat-producing clover was available at this time and it was not possible to determine whether the loss of the effect of penicillin on the fermentation pattern meant that the antibiotic was no

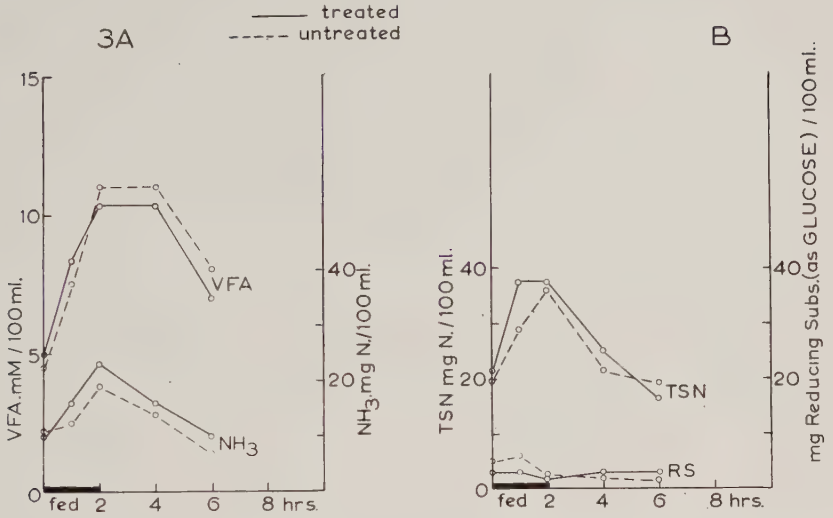


Fig. 3.—The effect of 200,000 units of procaine penicillin on rumen fermentation after repeated dosing with penicillin. A. volatile fatty acid and ammonia levels. B. total soluble nitrogen and reducing solutions (R.S.).

longer giving protection from bloat. It was our impression, however, that the treated animal still showed less foam in its rumen than the control animal. The colour difference of the rumen contents of the two animals had disappeared.

It seemed probable that the microbial flora of the treated animal had become resistant to the effect of penicillin, either by the production of resistant strains of bacteria normally present, or by a change in the balance of the flora. To make certain that a change in the microbial flora of both animals had not taken place, independent of the penicillin treatment, the animals were reversed for dosing. The animal that had acted as control in previous experiments was dosed with 200,000 units of penicillin and the marked difference in fermentation pattern between treated and untreated animals was again observed.

No further dosing of penicillin was carried out on these animals for two months. The animal that had become resistant to the action of penicillin was then dosed with 200,000 units of penicillin. It will be seen from the results shown in Fig. 4 that the animal has regained its sensitivity to penicillin.

From the results presented in Fig. 2 and Table 2, it was observed that there was a fall in rumen pH at 2 h after feeding even though there was practically no change in volatile fatty acid concentration. This indicated that some non-volatile acid was accumulating. The results in Fig. 4 show that it was probably lactic acid.

Identification of Reducing Substances

Owing to the number of analyses being carried out on each sample taken during the feeding experiments, insufficient clear rumen liquor

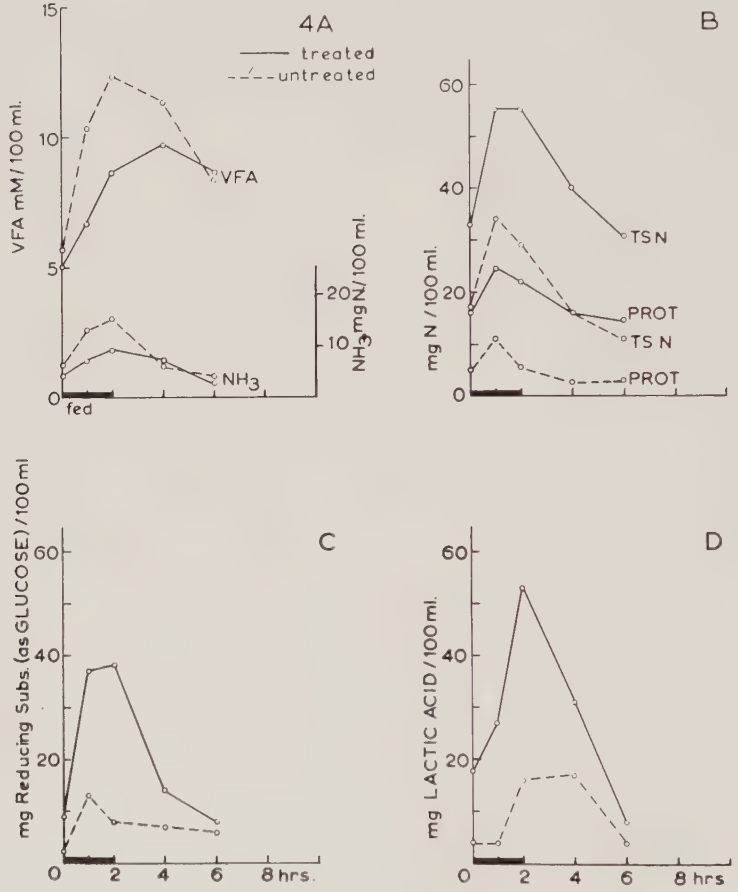


Fig. 4.—The effect of dosing 200,000 units of procaine penicillin to an animal that had become resistant to the antibiotic and then left untreated for two months. A. volatile fatty acids and ammonia. B. protein and total soluble nitrogen. C. reducing substances. D. lactic acid.

was available for a detailed study of the components of the fraction estimated as reducing substances. However, the paper chromatograms were run both on the 2 h rumen sample (20 mg reducing substances/100 ml) from an animal which had been previously dosed with 200,000 units of penicillin, and on the sample from the control animal. The solution from the control animal showed a single trace component which appeared to be chromatographically identical with arabinose. The solution derived from the penicillin-treated animal showed at least six reducing components. Three of these were chromatographically similar to arabinose, glucose, and galactose. Two possible reducing oligosaccharide spots (R_G = 0.62 and 0.33) were present. A fast-moving component (R_G 1.20) was also prominent. This component moved at the same rate as fucose and gave with aniline hydrogen phthalate the

brown stain typical of hexoses and methyl pentoses. It was clearly not mannose, rhamnose, xylose, or ribose.

Disappearance of Penicillin from the Rumen

The rate of disappearance of penicillin from the rumen, after administration through the fistula, was tested at three dose rates and the results are shown in Table 3.

TABLE 3. LEVEL OF PENICILLIN IN THE RUMEN AT VARIOUS TIMES AFTER DOSING

Time (h)	Penicillin (units/ml)		
	Dose 100,000	500,000	200,000
0	0	0	0
1	1	7	1
2	1	7	0.5
4	0.5	3	0.05
6	0.1	1	0
24	0	0	0

DISCUSSION

The results in Table 1 demonstrate once again how similar identical twins are in their behaviour and how useful they can be for this type of work.

Foaming Properties

From the point of view of bloat, the most striking comparison between treated and untreated animals was the marked difference in foaming properties of the rumen fluid. It will be noted in Table 2 that the foam expansions and foam stabilities of the crude rumen liquor from the penicillin-treated cow were very low compared with those of the control animal, which showed signs of moderate bloat. This is in spite of the fact that the treated animal's rumen liquor had a very high soluble-protein content compared with that of the control. Mangan (1959) has shown that solutions containing only 10 mg protein-N/100 ml form foams with considerable strength.

By contrast, the *centrifuged* rumen contents from the treated cow showed for the one- and two-hour samples the maximum foam expansion measurable in the apparatus and a very high foam strength. The 0 h sample would not be expected to show strong foaming properties as the pH was too high (Mangan 1958). This very great increase in strength of the foam produced from the centrifuged rumen liquor of the penicillin-treated animal (in agreement with the high soluble-protein content), as compared with strength of foam from the crude liquor, indicates that a strong anti-foaming agent was present and that it is removable by centrifuging. This increase in foam strength after centrifuging was not obtained on tests in the case of the control animal.

It has been found previously (Mangan 1958) that the plant chloroplasts, with their envelope of lipid, have anti-foaming properties, and it was suggested they may play an important part in the aetiology of bloat. It is postulated from the present results that the chloroplasts in the rumen of the penicillin-treated cow have much stronger anti-foaming action than in the untreated animal. This may be due to the inhibition of bacteria which normally modify the chloroplast lipid in some way to lessen its anti-foaming activity, e.g. probably by hydrogenation or by the action of microbial lipase.

If the foaming properties of the rumen liquor have been due to any capsulated slime-producing bacteria, centrifuging should presumably have reduced the foam properties of the rumen liquor. Cell-free rumen liquor at the end of feeding (2 h) contained, irrespective of bloat or treatment, 2–4 mg of polysaccharide/100 ml of crude rumen liquor (Bailey in preparation).

The results from the analyses of rumen constituents show a general slowing down of the rate of volatile fatty acid and ammonia production with a consequent piling up of fermentation intermediates. Gas production from fermentation is associated mainly with the formation of volatile fatty acids and would consequently have been reduced. This probably resulted in the rumen becoming less highly reducing than normal, giving rise to the brown-coloured rumen contents while the penicillin dosing was effective.

There was little difference between the pH values of the rumen contents of treated and untreated animals, as lactic acid accumulated in place of the volatile fatty acid normally produced. Hence pH was not a factor in the reduced foam formation in treated animals.

Effect of Fermentation

Owing to the intense activity of the microbial population, it is difficult to measure directly the rate of release of readily fermentable substances from plant material into the rumen or study the intermediate steps in their fermentation. The present results indicate that the use of antibiotics such as penicillin can be of use in slowing down particular steps so that some of the substrates and intermediates may accumulate temporarily. These will be considered in turn.

Reducing Substances. It is realised that the copper reduction method employed for measuring reducing substances is not specific for reducing sugars and that other non-sugar reducing substances may interfere (Heald 1951). Reid (pers. comm.) in Australia has found that tyrosine may account for rumen levels of "glucose" up to 10 mg per cent on high-protein diets, while phenylalanine is also determined by copper reducing method. However, chromatographic analysis of the amino fraction indicated that our samples contained no phenylalanine and that tyrosine was present only in low concentrations. The main amino acids present were alanine and γ aminobutyric, with lesser amounts of aspartic, glutamic and glycine (Mangan unpublished).

Reducing sugars were detected chromatographically in the 2 h sample from a penicillin-treated animal. From the intensity of the spots

it seems reasonable to assume that part, at least, of the fraction reported as reducing substances, is in fact composed of free reducing sugars. Most of the components detected were not present in a similar sample from the untreated animal.

The mixture of sugars detected does not appear to bear any clear relationship to the mixture of sugars found in red-clover extracts. These latter include xylose, glucose, fructose, sucrose, fructosyl-fructose, fructosyl-glucose and an isomer of raffinose; (Bailey 1958). If the fast component (R_G 1.20) is in fact fucose then the two main sugar components of bovine salivary mucoprotein, fucose and galactose (Gottschalk 1956) have apparently accumulated in the rumen of the penicillin-treated animal.

Lactic acid is found in the rumen liquor of normal animals only in trace amounts (Robinson *et al.* 1955). However, Elsden and Phillipson (1948) suggested that the appearance of lactic acid as an intermediary in the decomposition of glucose, both *in vivo* and *in vitro* indicated on the one hand the presence in the rumen of lactic acid bacteria and on the other the presence of lactic acid decomposing organisms. A number of species of each type have since been isolated (Oxford 1958). It appears probable that most of the propionate arises by lactate fermentation. The present results (Table 3) tend to confirm that lactate is an intermediate in volatile fatty acid formation and indicate that the organisms responsible for the fermentation of lactate are in the main much more sensitive to penicillin than are the lactate-producing types. These latter include holotrich protozoa which are known to be insensitive to penicillin.

Protein Breakdown. Penicillin treatment markedly inhibited protein metabolism in the rumen. In Figs. 1, 2, and 4 it will be seen that the treated animal had considerably more soluble nitrogen in the rumen than the control even before feeding. This was due in the main to higher levels of protein and animo nitrogen. It was suggested by Ferguson and Terry (1955) that plant protein would not be released rapidly enough into the rumen to contribute to bloat which can be characterised by a very rapid onset. It can be seen in Fig. 2, however, where 500,000 units of penicillin were given, the protein level rose very rapidly in the first hour and reached 50 mg N/100 ml in 2 h, indicating a rapid release of soluble plant proteins. Other results have shown that the salivary mucoprotein constitutes a maximum of 15% of the soluble protein level in the rumen.

The amino acid pattern closely resembled that of free amino acids in the clover rather than that found in the clover protein (Mangan unpublished). This indicated that the initial rise in amino nitrogen was due in these experiments to the release of the non-protein nitrogen compounds in the clover. After the 2 h sample, the rise in amino nitrogen accompanies protein breakdown.

CONCLUSIONS

It appears that penicillin owes its effectiveness in bloat control partly to its action in depressing gas production and partly to an

undetermined inhibitory effect on the process of modification by microbial action of chloroplast fat. In its native state this fat can act as an anti-foaming agent. Penicillin is thus a useful tool in helping study the role of this anti-foaming agent in the aetiology of bloat. This is being further investigated. It seems likely that penicillin would be effective as a prophylactic in bloat control only for a certain number of doses as resistance to its effect soon develops. However it is seen that animals left untreated for a period recover their sensitivity to its action.

Acknowledgements

Our thanks are due to Mr. G. A. Cox of the Dairy Research Institute (N.Z.) for carrying out the penicillin assays and to Misses M. E. Brown and J. Michael and to Mr. P. Vlieg for their technical assistance.

References

Andrews, E. D.; Gall, L. S.; Hart, L. I. 1957a: *N.Z. J. Sci. Tech. A38:* 649.
Andrews, E. D.; Gall, L. S.; Hart, L. I.; Anderson, J. P. 1957b: *Ibid. A38:* 654.
Bailey, R. W. 1958: *J. Sci. Fd. Agric. 9:* 748-53.
Barrentine, B. F.; Shawver, C. B.; Williams, L. W. 1956: *J. Anim. Sci. 15:* 440.
Chibnall, A. C.; Rees, M. W.; Williams, E. F. 1943: *Biochem. J. 37:* 354.
Conway, E. J. 1947: "Micro-diffusion Analysis and Volumetric Error." Crossley, Lockwood, London.
Doak, B. W. 1939: *N.Z. J. Sci. Tech. B21:* 90.
Elsden, S. R.; Phillipson, A. T. 1948: *Annu. Rev. Biochem. 17:* 705.
Ferguson, W. S.; Terry, R. A. 1955: *J. agric. Sci. 46:* 257.
Friedemann, T. E.; Graeser, J. B. 1933: *J. biol. Chem. 100:* 291.
Gottschalk, A.; Ada, G. L. 1956: *Biochem. J. 62:* 681.
Hardie, W. B.; Downing, H. E.; Reynolds, N. M.; Luther, H. G. 1953: *Antibiotics Annu. 1953-54.* p. 372.
Heald, P. J. 1951: *Brit. J. Nutr. 5:* 75.
Huhtanen, C. N.; Saunders, R. K.; Gall, L. S. 1954: *J. Dairy Sci. 37:* 328.
Hungate, R. E.; Fletcher, D. W.; Dougherty, R. W.; Barrentine, B. F. 1955: *Appl. Microbiol. 3:* 161.
Jermyn, M. A.; Isherwood, F. A. 1949: *Biochem. J. 44:* 402.
Johns, A. T. 1954: *N.Z. J. Sci. Tech. A36:* 289.
Johns, A. T.; Mangan, J. L.; Reid, C. S. W. 1957: *N.Z. vet. J. 5:* 115.
Johns, A. T. 1958: *Vet. Rev. Annot. 4 (1):* 17.
Mangan, J. L. 1958: *N.Z. J. agric. Res. 1:* 140.
———1959: *Ibid. 2:* 47.
McDonald, I. W. 1948: *Biochem. J. 42:* 584.
Oxford, A. E. 1958: *N.Z. Sci. Rev. 16:* 38.
Partridge, S. M. 1949: *Nature, Lond. 166:* 443.
Robinson, R. Q.; Doetsch, R. N.; Sirotnak, F. M.; Shaw, J. C. 1955: *J. Dairy Sci. 38:* 13.
Shaffer, P. A.; Hartmann, A. F. 1921: *J. biol. Chem. 45:* 365.
Trevelyan, W. E.; Procter, D. P.; Harrison, J. S. 1950: *Nature, Lond. 166:* 444.
Van Slyke, D. D. 1929: *J. biol. Chem. 83:* 425.
Wasserman, R. H.; Duncan, C. W.; Churchill, E. S.; Huffman, C. F. 1952: *J. Dairy Sci. 35:* 371.
Whitehead, H. R.; Cox, G. A. 1958: *J. appl. Bact. 19:* 247.

BLOAT IN CATTLE

XIV. THE CONCENTRATION OF SOLUBLE POLYSACCHARIDE IN THE RUMEN CONTENTS OF COWS FED ON RED CLOVER

By R. W. Bailey, Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 5 December 1958*)

Summary

Crude rumen liquor from cows fed on red clover was fractionated in order to separate and estimate soluble polysaccharides, plant and protozoan starch, and bacterial capsular polysaccharides. The animals used included a pair of identical twins under both bloating and non-bloating conditions.

The concentration of polysaccharide in cell-free rumen liquor was low and no greater in bloating than non-bloating animals.

The hot-water extract of the solids centrifuged out of the crude rumen liquor yielded a fraction which, in non-bloating animals, showed a considerable increase on feeding. In animals which bloated, the initial level was higher than in non-bloating animals but showed no marked increase after feeding. Control of bloat by penicillin administration also prevented an increase in this fraction.

The concentration of capsular polysaccharide in the fraction obtained by an acetic acid decapsulation technique applied to the centrifuged solids from rumen liquor, showed no correlation with bloat incidence.

Much of the polysaccharide present in the isolated fractions was starch, probably of the amylopectin type. Positive tests for dextran were not obtained in any of the fractions.

Introduction

The formation of a stable foam in the rumen has been established as the causative agent of bloat in cattle feeding on pasture legumes (Johns 1954). Polysaccharide slimes or gums produced by rumen bacteria have been suggested as one of the possible foaming or foam stabilising agents involved in the formation of the foam (Hungate *et al.* 1955). However, published results on the nature and amount of soluble polysaccharide in cattle rumen, particularly during bloat, are scarce, and the connection between polysaccharides and bloat has not been determined.

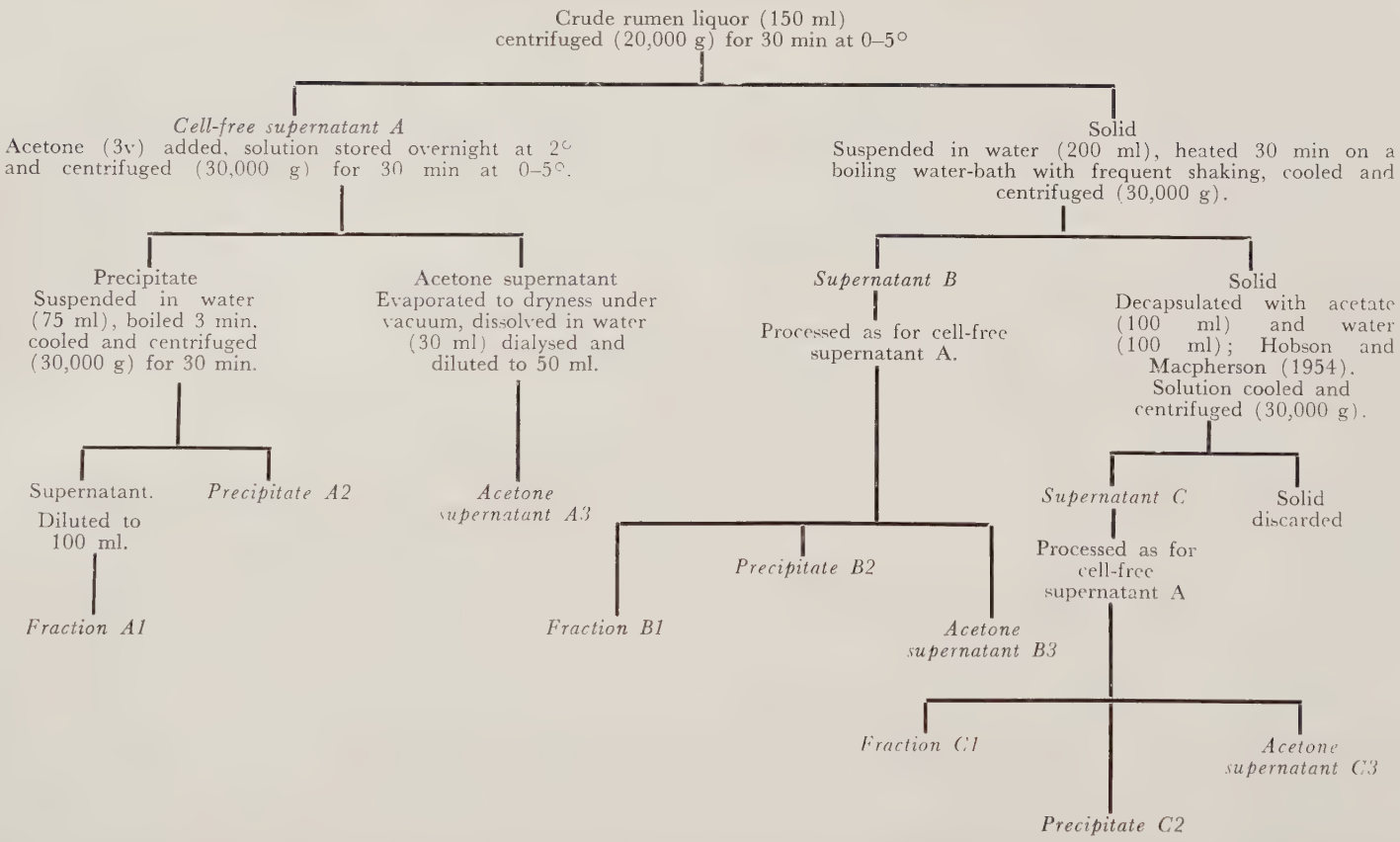
Samples of rumen contents have been obtained both from cows suffering from various degrees of bloat and from cows without bloat or in which the bloat had been prevented by oil or penicillin treatment. This paper reports the results obtained during the 1957-58 season.

Experimental

Rumen Liquor Samples

Samples of rumen liquor were obtained from fistulated identical-twin cows which were feeding on freshly cut red clover. Details of the

Fig. 1.– Fractionation of Crude Rumen Liquor



feeding system and also of the grading scheme used for assessing bloat in the cows are given by Johns (1954). Samples were taken (a) immediately before feeding (0 h), and (b) at the end of feeding (2 h). Generally the animals consumed between 25 and 40 lb of red clover (fresh wt) during the 2 h feed. They had been on a red-clover diet for at least 2 days before any particular feeding experiment. When necessary, to counteract bloating, the cows were drenched with peanut oil by the method of Reid and Johns (1957). The technique used to control bloat with penicillin has been described by Mangan *et al.* (1959).

At each sampling a bulk sample (0.5–1 gal) of rumen contents was taken and squeezed through muslin. A portion of the filtrate (crude rumen liquor) was immediately cooled and fractionated. Rumen samples from bloated cows gave, on squeezing through the muslin, very frothy filtrates. These filtrates were allowed to stand at 0–5° for 30 min in order to obtain sufficient liquor.

Fractionation of Rumen Liquor

The fractionation of the rumen liquor samples is described in detail in the scheme (Fig. 1) shown below.

In addition to an examination of polysaccharides in solution, two extracts were prepared from the residue of microbial and plant cells which had been centrifuged out of the crude rumen liquor. The first of these, a hot-water extract, was expected to contain the less soluble polysaccharides; while the second, an acetic acid extract, had been shown to dissolve the capsular polysaccharides of rumen bacteria (Hobson and Macpherson 1954). It was considered possible that the encapsulated bacterial cells might influence the foaming properties of rumen contents. The two extracts would also be expected to contain soluble polysaccharides from protozoa and plant cells disrupted during the extraction process. Each rumen sample was fractionated to the stage of adding acetone to the three main supernatants (A, B, and C; Fig. 1) immediately after sampling. Only a limited number of samples (maximum of four) could, therefore, be dealt with from any particular feeding experiment. After analysis for polysaccharide content the fractions were concentrated to a small volume (10–20 ml) under vacuum and freeze-dried for subsequent chromatographic examination.

Measurement of Polysaccharide

The polysaccharide content of the final aqueous solutions (Fractions A1, B1, and C1; acetone supernatants A3, B3, and C3: Fig. 1) was measured using an anthrone method which had been found suitable for measuring hexoses and methyl pentoses (Bailey 1958a), but which did not measure pentoses. Interference from any ribose associated with proteins was, therefore, eliminated. The three precipitates (A2, B2, and C2; Fig. 1) were suspended in water (10–20 ml), heated at 100°C. for 6 h with an equal volume of 2N H_2SO_4, and filtered. The hexose content of the filtrates was measured by the same anthrone method. A preliminary examination of hydrolysates of the various fractions showed that glucose was the major sugar component

in all of the fractions. Glucose was used as the standard sugar in the anthrone method and all results were reported as mg of anhydro-glucose per 100 ml of original crude rumen liqour.

Hydrolysis of Fractions

(a) *Acid Hydrolysis.* Freeze-dried solid (10–20 mg) in N H_2SO_4 (2 ml) was heated for 4 h at 100°C. The hydrolysates were neutralised with solid $BaCO_3$, filtered and concentrated to a small volume (0.2 ml) for chromatographic analysis. Fractions were also hydrolysed by heating them, under the same conditions, in N HCl. After evaporation to dryness in a vacuum desiccator over solid NaOH the residues were dissolved in water (0.2 ml).

(b) *Enzymic Hydrolysis.* Freeze-dried solid (20–50 mg) was dissolved in acetate buffer (2 ml, 0.5M, pH 5.5) and enzyme solution (1 ml, amylase or dextranase) added. The solutions, together with appropriate controls, were incubated for 24 h at 37°C. under toluene. The final digests were desalted with Dowex 50 and Dowex 2 ion-exchange resins and concentrated to a small volume (0.2 ml).

Paper Chromatographic Analysis

Paper chromatograms of acid and enzymic hydrolysates were developed with one of the following solvent mixtures:

(a) The top layer of a mixture of water, n-butanol, ethanol, and ammonia, d.0.88 (40, 49, 10, and 1% v/v; Baker *et al.* (1954).

(b) The top layer of a mixture of ethyl acetate, water, and pyridine (2:2:1 v/v; Jermyn and Isherwood 1949).

(c) A modified version of solvent (b) which was more suitable for resolving oligosaccharides (Malpress and Hytton 1958). This solvent was used for the analysis of dextranase digests.

Sugars and oligosaccharides were detected on the papers with sprays of silver nitrate (Trevelyan *et al.* 1950) and aniline hydrogen phthalate (Partridge 1948).

Enzymes

β-amylase. A crude extract of β-amylase was prepared from New Zealand sweet potatoes by the first part of the method of Balls *et al.* (1948).

Dextranase. A dextranase preparation was obtained from a culture of *Lactobacillus bifidus* (Bailey and Clarke in press). This enzyme hydrolysed dextran, both branched and unbranched molecules, to a mixture of oligosaccharides; principally isomaltotriose, isomaltotetrose and isomaltopentose.

Results

Distribution of Polysaccharide Among the Fractions Obtained from Rumen Liquor

Any polysaccharide present in the three initial extracts (supernatants A, B, and C; Fig. 1) was expected to be precipitated with

acetone and to appear in fractions A1, B1 and C1 (Fig. 1). On a number of occasions all nine fractions obtained from a rumen liquor sample were analysed for polysaccharide. Typical results obtained are listed in Table 1.

TABLE 1. DISTRIBUTION OF POSSIBLE POLYSACCHARIDES AMONG RUMEN LIQUOR FRACTIONS

Samples obtained from cow I fed on red clover, bloated to grade II and oil-drenched before 2 h sample (Exp. 5, Table 2).

Sampling Time (h)	Polysaccharide as Anhydro-glucose (mg/100 ml of crude rumen liquor)								
Fractions	A1	A2	A3	B1	B2	B3	C1	C2	C3
0	5.5	1.4	1.0	29.0	2.0	1.5	11.3	1.6	0.5
2	2.9	2.1	0.4	30.0	3.1	2.2	30.0	2.2	1.4

Of the possible polysaccharide in supernatant A (fractions A1, A2, and A3; Fig. 1) quite a large proportion occurred in fraction A2. This was not unexpected as this fraction, which presumably consisted largely of denatured protein, would contain any denatured salivary mucoprotein (containing galactose and fucose: Gottschalk 1956) present in the original rumen liquor. Hexoses measured in this fraction were not considered to be true polysaccharide. The other precipitates (B2 and C2; Fig. 1) and the final acetone supernatants (A3, B3, and C3; Fig. 1) contained only low amounts of possible polysaccharide compared with their respective main fractions (A1, B1, and C1). It was concluded that these latter three fractions did in fact contain most of the true polysaccharide present in the three original extracts (supernatants A, B, and C).

Polysaccharide Content of Fractions from the Rumen Contents of Cows under Various Conditions of Bloat

Polysaccharides were measured in the three main fractions (A1, B1, and C1) obtained from a number of rumen samples. Samples were taken from cows under conditions of no bloat and various degrees of bloat. Samples were also taken from a cow in which bloat had been prevented by penicillin treatment and from a cow in which bloat had been reduced by oil-drenching before sampling. The results obtained are listed in Table 2.

For comparison three rumen samples were obtained from the cows during a period when they were being fed on freshly cut ryegrass. Results obtained from these samples are also listed in Table 2.

Sugars Present in Rumen Fractions A1, B1, and C1

Acid hydrolysates of the freeze-dried fractions (Exp. 1–5; Table 2) were analysed for sugars by paper chromatography. In all of the fractions a component chromatographically identical with glucose repre-

TABLE 2. CONCENTRATION OF POLYSACCHARIDE IN FRACTIONS FROM THE RUMEN CONTENTS OF CATTLE

Cows fed freshly cut red clover except where otherwise stated.

Experiment No.	Date (1957)	Cow No.	Grade of Bloat	Treatment	Sampling Time (h)	Polysaccharide as Anhydro-glucose (mg/100 ml of crude rumen liquor) Fractions A1*	B1*	C1*
(a) No bloat during feeding								
1	Nov. 13	I	—	—	0	3.8	13.0	4.5
			±		2	1.8	38.0	15.0
2	Nov. 21	293	—		0	7.0	22.5	8.2
			—		2	2.5	63.0	17.4
	Nov. 21	294	—	Penicillin	0	7.8	15.3	5.6
			—		2	4.1	71.0	24.6
(b) Bloat occurring during feeding								
3	Oct. 9	293			0	9.0	36.0	6.9
			II		2	2.2	36.9	17.1
		294		Penicillin	0	4.4	29.2	5.4
			—		2	0.9	26.0	9.0
4	Oct. 30	293			0	5.1	17.0	3.5
			III		2	3.0	49.2	17.6
5	Nov. 1	293			0	6.9	30.3	6.2
			II-III		2	2.4	40.0	18.0
				Oil drenched before 2 h sample				
		I			0	5.5	29.5	11.3
			II		2	2.9	30.5	30.0
(c) Cows fed on freshly cut ryegrass								
6	Sept. 18	293	—		0	6.0	55.0	7.6
	Sept. 18	I	—		0	4.3	28.0	6.8
7	Sept. 30	293	—		2	5.4	90.0	31.6

* A1 = Polysaccharide in solution.

B1 = Chiefly plant and protozoan starch.

C1 = Capsular polysaccharide.

sented at least 80–90% (visual estimation) of the possible sugar spots present on the papers. No other sugars were detected in hydrolysates of the A1 (Fig. 1) fractions. In addition to the intense glucose spot, B1 hydrolysates showed trace components corresponding to galactose, fructose, rhamnose, xylose and ribose, while the C1 hydrolysates showed trace components corresponding to galactose, fructose and rhamnose. Uronic acids and amino-sugars were not detected on the papers.

Polysaccharides Present in Rumen Fractions A1, B1, and C1

Preliminary tests were made in order to identify, as far as possible, the polysaccharides present in the fractions. With iodine solution (0.1N) all of them (Exp. 1–5; Table 2) gave a brownish-violet colour which was typical for amylopectin, and in particular for protozoan starch, which is amylopectin (Oxford 1951). When the fractions were incubated with β-amylase and the final digests analysed for sugars by paper chromatography components corresponding to glucose (trace) and maltose (intense spot) were detected. Starch, probably amylopectin, is evidently a major component in all of the fractions.

The various fractions were also incubated with dextranase but no oligosaccharides indicative of dextran could be detected on paper chromatograms of any of the digests.

DISCUSSION

Only a limited number of rumen samples have been fractionated. The results obtained however appear to be reasonably consistent and suggest the following conclusions.

The amount of soluble polysaccharide present in cell-free rumen liquor (fraction A1, Table 2) is low. Even when the other possible hexose polymer associated with the cell-free supernatant (fractions A2 and A3, Table 1) was included, the total possible polysaccharide in solution only once (Exp. 3, Table 2) exceeded 10 mg/100 ml of crude rumen liquor. Comparison of results from 0 to 2 hour samples shows, always, a marked drop in the amount of fraction A1 after feeding, possibly due to dilution of the rumen contents with saliva and plant juices. It is interesting to note that Mangan (1959) has shown that cell-free rumen liquor, obtained by the same centrifuge process, foams readily to give a very stable foam. Its foaming properties become stronger during the course of feeding, i.e. while the concentration of polysaccharide in the cell-free liquor is decreasing. There appears, also, to be no difference between the concentrations of fraction A1 in the rumen contents of cows on bloating and non-bloating diets.

The hot-water extracts (fractions B1) contain the most polysaccharide. Samples taken at 0 h from cows on bloat-producing clover diets (Exp. 3–5, Table 2) with one exception (Exp. 4), contain much more of this fraction than do 0 h samples from cows on non-bloating clover diets. However, while the 2 h samples from the bloating cows show little change in the concentration of this fraction, those from the non-bloating cows show a threefold increase in concentration.

A possible explanation of this difference may be on a less uniform distribution of certain kinds of micro-organisms between the foam and the liquid settling out from it. This could lead to a sampling error with the very frothy samples from bloating cows when compared with the less frothy samples from non-bloating cows. Only the liquid part was analysed in this study. This point merits further investigation.

The concentrations of the C1 fractions, containing any capsular polysaccharide, appear to be independent of the bloatiness of the clover. In all cases there is a rise in the concentration of this fraction in the 2 h as compared with the 0 h samples.

In one case where penicillin had controlled bloat (Exp. 3, cow 294, Table 2) the concentration of each fraction was lower than in the identical twin with bloat. Results of Mangan *et al.* (1959) indicate that penicillin lowers the activity of rumen bacteria. Lower levels of polysaccharide would, therefore, be possible in the penicillin-treated animal.

Iodine and amylase tests indicate that starch is a major component of the three fractions. This is not unexpected, as rumen protozoa (Oxford 1951; Masson and Oxford 1951), rumen bacteria (Hobson and Mann 1955), and clover leaves (Bailey 1958b), have all been shown to contain starch. Weller *et al.* (1954) have in fact suggested that two thirds of the starch present in sheep rumen contents is in the protozoa. Tests carried out in this laboratory have shown that nearly all the iodophilic storage polysaccharide within the holotrich ciliates, together with a substantial part of the corresponding storage material of the oligotrich ciliates, which were chiefly epidinia in these instances, are in fact brought into solution by the hot water treatment employed to obtain fraction B1. It is, however, unlikely that any intracellular bacterial starch would be so extracted. Since rumen ciliate protozoa are not affected by penicillin at the levels used, there can be no surprise at the apparent failure of the antibiotic markedly to change the B1 levels although lowering the 2 h C1 level when preventing bloat (see results for penicillin-treated animal and its twin on 9 October in Table 2).

Results reported by Bailey and Oxford (1958a, b) on the conversion of sucrose to dextran by rumen strains of *Streptococcus bovis* suggest that this soluble polysaccharide might be present in the rumen contents when the animal's diet is rich in sucrose. Fresh red clover grown at this laboratory contains 2.5–3.5% (dry wt) of sucrose (Bailey 1958c). Dextran has not, however, been detected in any of the fractions so far isolated. Its occurrence in the rumen may be transient and difficult to demonstrate as strains of *L. bifidus* isolated from the rumen (Clarke, in press) have been shown to secrete a dextranase (Bailey and Clarke, in press).

Apart from the 0 h levels of hot-water-soluble polysaccharide there does not appear to be any correlation between bloat in the cows and the concentration of soluble polysaccharides in the rumen contents, at least at the beginning and end of feeding. Mangan's (1959) finding that the foaming properties of crude rumen liquor increase with centrifuga-

tion cast doubt on the possible connection between bloat and rumen polysaccharides, particularly when considered in conjunction with the results reported here. It is of course possible that any bacterial polysaccharides, e.g. dextran, connected with bloat may have only a short existence in the rumen. Sampling at short intervals during the development of bloat might give indications of a positive connection between bloat and rumen polysaccharides. However, under the feeding regime employed at this laboratory, bloat is usually at a maximum about 2 h after the commencement of feeding, hence this time was chosen for sampling.

ACKNOWLEDGEMENTS

The assistance of Mr. C. S. W. Reid and Mr. P. Vlieg of this laboratory in taking the rumen samples is gratefully acknowledged. This work was carried out with the technical assistance of Miss J. Michael.

REFERENCES

Bailey, R. W. 1958a: The Reaction of Pentoses with Anthrone. *Biochem. J. 68 (4):* 669-72.

———1958b: Carbohydrates in Pasture Species. I. The Starch Content of Clovers and Ryegrass. *J. Sci. Fd. Agric. 9:* 743-7.

———1958c: Carbohydrates in Pasture Species. II. The Soluble Sugars of Red Clover. *Ibid. 9:* 748-53.

Bailey, R. W.; Oxford, A. E. 1958a: Pre-requisites for Dextran Production by *Streptococcus bovis. Nature, Lond., 182:* 185.

———1958b: A Quantitative Study of the Production of Dextran from Sucrose by Rumen Strains of *Streptococcus bovis. J. gen. Microbiol. 19:* 130.

Bailey, R. W.; Clarke, R. T. J. (In press): A Bacterial Dextranase. *Biochem J.*

Balls, A. K.; Walden, M. K.; Thompson, P. R. 1948: A Crystalline β-Amylase from Sweet Potatoes. *J. biol. Chem. 173:* 9.

Barker, S. A.; Bourne, E. J.; Bruce, G. T.; Neely, W. B.; Stacey, M. 1954: The Structure of a *Betacoccus arabinosaceous* Dextran. *J. chem. Soc.:* 2395.

Clark, R. T. J. (In press): A Dextran-fermenting Organism from the Rumen closely Resembling *Lactobacillus bifidus. J. gen. Microbiol.*

Gottschalk, A.; Ada, G. L. 1956: The Separation and Quantitative Determination of the Component Sugars of Mucoproteins. *Biochem. J. 62:* 681.

Hobson, P. N.; Macpherson, M. J. 1954: Some Serological and Chemical Studies on Materials Extracted from an Amylolytic *Streptococcus* from the Rumen of the Sheep. *Ibid. 57:* 145.

Hobson, P. N.; Mann, S. O. 1955: Some Factors Affecting the Formation of Iodophilic Polysaccharide in Group D *Streptococci* from the Rumen. *J. gen. Microbiol. 13:* 420.

Hungate, R. E.; Fletcher, D. W.; Dougherty, R. W.; Barrentine, B. F. 1955: Microbial Activity in the Bovine Rumen: Its Measurement and Relation to Bloat. *Appl. Microbiol. 3:* 289.

Jermyn, M. A.; Isherwood, F. A. 1949: Improved Separation of the Sugars on the Paper Partition Chromatogram. *Biochem. J. 44:* 402.

Johns, A. T. 1954: Bloat in Cattle on Red Clover, I. *N.Z. J. Sci. Tech. A36:* 289.

Malpress, F. H.; Hytton, F. E. 1958: The Oligasaccharides of Human Milk. *Biochem. J. 68:* 708.

Mangan, J. L. 1959: Bloat in Cattle. XI. The Foaming Properties of Proteins, Saponins, and Rumen Liquor. *N.Z. J. agric. Res. 2:* 47.

Mangan, J. L.; Johns, A. T.; Bailey, R. W. 1959: Bloat in Cattle. XIII. The Effect of Orally Administered Penicillin on the Fermentation and Foaming Properties of Rumen Contents. *Ibid. 2:* 342-54.

Masson, F. M.; Oxford, A. E. 1951: The Action of the Ciliates of the Sheep's Rumen upon Various Water Soluble Carbohydrates Including Polysaccharides. *J. gen. Microbiol. 5:* 664.

Oxford, A. E. 1951: The Conversion of Certain Soluble Sugars to a Glucosan by *Holotrich* Ciliates in the Rumen of Sheep. *Ibid. 5:* 83.

Partridge, S. M. 1949: Aniline Hydrogen Phthalate as a Spraying Reagent for Chromatography of Sugars. *Nature, Lond., 164:* 443.

Reid, C. S. W.; Johns, A. T. 1957: Bloat in Cattle on Red Clover. III. Treatment and Prevention with Antifoaming Agents. *N.Z. J. Sci. Tech. A38:* 908.

Trevelyan, W. E.; Procter, D. P.; Harrison, J. S. 1950: Detection of Sugars on Paper Chromatograms. *Nature, Lond., 166:* 444.

Weller, R. A.; Gray, F. V. 1954: The Passage of Starch Through the Rumen of the Sheep. *J. exp. Biol. 31:* 40.

BLOAT IN CATTLE

XV. FURTHER OBSERVATIONS CONCERNING THE CILIATE *Epidinium ecaudatum*, AN INHABITANT OF THE RUMENS OF COWS LIABLE TO LEGUME BLOAT

By A. E. Oxford, Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 11 December 1958*)

Summary

Epidinium ecaudatum can slowly metabolise glucose, and to a lesser extent fructose, with production of amylopectin storage granules characteristically situated. The non-ionic "Pluronic" detergent L62, a mixed polymer of ethylene and propylene glycols, is lethal to *Epidinium* above a concentration of 0.05%. It is less harmful to the larger rumen holotrichs particularly when the latter are not well filled with storage polysaccharide. Introduction of L62 (25–75 ml) into the cow's rumen quickly brings about a diminution in the active ciliate population. Epidinia and the smaller rumen holotrichs seem most affected.

Introduction

In Part IX of this series (Oxford 1958) it was shown that *Epidinium ecaudatum* was the predominant oligotrich ciliate protozoon in the rumens of several cows fed exclusively on red clover, and that this ciliate ingests and digests red clover starch grains and chloroplasts with great avidity. A study of its powerful α-amylase has been published (Bailey 1958); its lipase is also being investigated in this laboratory (Wright in preparation). Since *Epidinium* may possibly be of some importance in determining the onset of clover bloat, further studies have now been made with this ciliate in order to discover: (a) improved methods of obtaining clean washed suspensions of the living ciliate at various times in the host's feeding cycle, not merely 4–6 h after the morning feed as previously detailed; (b) whether glucose and/or fructose can possibly be metabolised by it with formation of fermentation products and storage polysaccharide; (c) the chemical nature of the *Epidinium* storage polysaccharide; and (d) the behaviour of *Epidinium* and other rumen ciliates in the presence of anti-foaming non-ionic detergents such as might be administered for the prevention of bloat. It is already known that anionic detergents, such as Teepol, are potent agents in killing and disrupting these ciliates (Masson and Oxford 1951), as is also any hydrocarbon-soluble neutral compound with slight solubility in water (e.g. menthol, c.f. Eadie *et al.* 1956). The particular class of neutral, non-ionic, surface-active agents recently under test as a bloat preventive at this station, viz complex straight-chain mixed polymers of ethylene and propylene glycols known as "Pluronics", have rather the reverse of this combination of properties. They have considerable water-, but not very great hydrocarbon-solubility; consequently their effect on the living rumen ciliates is of considerable theoretical as well as practical interest. This paper also includes an account of the

effect of administration of one such detergent (L62) upon the active ciliate population in the rumen of the cow so treated.

Methods

Unless otherwise stated, animals, rations, and experimental details were as in Part IX (Oxford 1958). All experiments were carried out during the spring months (October and November) of 1958.

Preparation of Washed Suspensions of Living Ciliates in Various Stages of Nutrition

(a) *The Mixed Culture, Chiefly Holotrichs and Epidinia in the Starved State.* In order to obtain such a culture, the rumen contents should be taken after overnight starvation. Since the strained rumen liquor so obtained, although often frothy, shows little gas evolution, the separation into three layers in the funnel does not readily take place (Oxford 1958). Gas generation in the rumen liquid to which buffer has been added readily occurred, however, when xylose (0.5%) was added, and a well defined bottom protozoan layer soon formed. Galactose may also be used for the same purpose. Only the bacteria rapidly attack xylose (or galactose) with gas evolution (Heald 1952). It is inadvisable to use glucose since the holotrich ciliates also attack it very rapidly thereby filling themselves with storage polysaccharide (Oxford 1951).

(b) *The Mixed Culture with the Epidinia Moderately Full of Starch and Chloroplasts.* It is inadvisable to attempt to get clean suspensions of full or moderately full ciliates by working up rumen contents 1–3 h after the morning feed of fresh red clover. Considerable amounts of finely divided green plant material then separate out with the protozoa and are not easily removed by the usual procedure of washing by decantation. Satisfactorily clean suspensions could sometimes be obtained but the final yield of ciliates was disappointingly small and was no indication of the actual rumen population at that time.

To obtain the desired culture under our feeding regime we found it best to proceed as in (a) above save that the cow normally fed exclusively on red clover was grazed on mixed pasture for two days.

(c) *Epidinia Only, in the Starved State.* Rumen contents were removed as in Part IX, i.e. 4–6 h after the morning feed. The subsequent processing with use of mannose to destroy holotrichs was exactly as described previously save that terramycin hydrochloride (0.01% in APB buffer) was substituted for neomycin sulphate, being a cheaper and more readily obtained antibiotic. The buffer also contained penicillin (0.003% or more) as before. The culture was not given clover starch or green plant material before overnight incubation with mannose.

(d) *Epidinia Only, in the Full or Moderately Full State.* This culture is difficult to arrive at. It is best to proceed as in (c), and to add a certain amount of clover starch containing some green plant

material (Oxford 1958) at the same time that mannose is added. The layer of protozoa on the bottom of the conical flask must not be too thick. Only experience can enable one to judge the right amount of food to add. If too much food is added, the culture although not completely moribund, may next morning contain too many dead epidinia to be of any immediate use for enzyme studies; if too little food is added, the culture may be healthy looking but too many individual epidinia will still be relatively empty.

The above methods of harvesting ciliates from strained rumen liquor are reasonably quantitative since hardly any more ciliates could be obtained by further processing of the green, scummy layer rising to the top in the separating funnel. Further, when the rumen liquor itself was very frothy, it was found that, weight for weight, the yield of ciliates from the froth, after settling, was much less than from the liquid under the froth.

Qualitative Study of Action of Epidinia on Glucose or Other Soluble Sugar

A starved 100 ml holotrich-free culture (usually prepared as described under (a) above) was well washed by decantation as early as possible next morning with APB buffer (Oxford 1958) containing antibiotics. It was then divided into two or more approximately equal parts, each contained in a nearly full 25-ml conical flask. The sugars (glucose, fructose; at 0.2% final concentration) was then added save to one flask which was kept as control. The closed flasks were incubated for 6 h with occasional shaking and the vigour, or otherwise, of the gas evolution noted. Before overnight incubation was continued, the supernatant in each flask was replaced by fresh APB buffer containing antibiotics and also the appropriate sugar, if any. Small specimens of the bottom ciliate layer in each flask were examined microscopically after 3, 6, and 24 h incubation. After low-power examination of the living ciliates under a cover slip, a drop of Lugol's iodine was added, the cover slip replaced and the extent and kind of iodophilic material in numerous epidinia was examined under the high dry objective.

In one experiment, the cultures were each given a very small supplementary feed of green plant material (GPM, see Part IX) containing practically no clover starch grains before overnight incubation.

Extraction of Starch from Ciliates

After incubation, the starch-like material in the centrifuged deposit of ciliates was extracted, either by the perchloric acid method of Pucher *et al.* (1948), or better, since a cleaner product is obtained, by heating with distilled water (4 ml) for 1 h at 100°C. The mixture, containing also some fine white sand, was stirred with a glass rod from time to time. The starch in the final clear supernatant was precipitated as the iodine complex in presence of 6% NaCl, and regenerated (usually 5–10 mg) by the action of alcoholic NaOH as described by Pucher *et al.* (1948). It was then dried *in vacuo* over concentrated H_2SO_4 and redissolved in distilled water (2 ml) by heating in a boiling water bath for a few minutes. The exact tint given with very dilute Lugol's iodine added gradually was then noted.

Mode of Testing of Effect of Pluronic Detergent on Epidinium *and other Rumen Ciliates*

Pluronic detergents L61 and L62* were tested, but most of the work was done with the latter which disperses much more easily in water. According to the makers it has a powerful anti-foaming effect, and is a large molecule of molecular weight of about 1,650, of which 20% consists of ethylene glycol residues bi-terminally situated, the central core of 80% being propylene glycol residues. The detergent, at various concentrations between 0.025% and 0.5% (w/v) was dispersed as completely as possible at 38°c. in APB buffer, in 50-ml conical flasks. Antibiotics and a well washed mixed culture of ciliates, prepared as described under (c) or (b) on p. 3, were added to give a layer just covering the bottom. Incubation was at 38°c. with gentle shaking from time to time. At intervals, small specimens of the bottom layer were withdrawn for microscopic examination. The degree of turbidity in the supernatant, indicating disruption of ciliates, was also noted. In one experiment, glucose (0.2%) was also added to the buffer as well as the detergent L62. Appropriate controls without detergent were put up in all instances.

Results

Behaviour of Epidinia Towards Glucose and other Hexoses

Preliminary experiments (Oxford 1958) seemed to show that epidinia did not metabolise glucose but it was not then determined whether the culture had, in fact, maintained itself on starch-containing particles contained in, or released from, dead ciliates. It is obviously desirable to start with a uniform culture of motile but reasonably empty epidinia, derived, for example, from the previous day's rumen liquor taken 5 h after the cow's morning feed of red clover: this culture should not have been fed plant starch overnight. When these precautions were observed, there was, in fact, no difficulty at all in demonstrating that epidinia can slowly metabolise glucose to yield not only gas, as revealed by disappearance of glucose and a build-up of pressure in the culture flask, but also abnormally large amounts of iodophilic storage polysaccharide. The best conditions for demonstrating this effect were an initial glucose concentration of 0.2% (in presence of the usual antibiotics) and an incubation time of 24 h. Under these conditions the control culture, without glucose or any other carbohydrate, was always completely moribund, containing very little iodophilic material. On the other hand, the corresponding culture with glucose still contained a majority of living organisms which *all* stained solidly brownish-purple with Lugol's iodine. Most of the storage polysaccharide was laid down in granules in the ectoplasmic region which, after 24 h, was often so greatly distended that it sagged at the anal end of the ciliate in the form of a separate bag full of granules. This was easily disrupted by mechanical pressure with release of the granules. The storage granules themselves were disc-like, resembling those of the holotrich ciliates previously described (Oxford 1951). On the average they seemed a little larger than the holotrich amylopectin granules, save for those in

* Wyandotte Chemicals Corporation, Wyandotte, Mich., U.S.A.

the tail of the ciliate which were considerably smaller. Extraction of the starch from such a 24 h culture of epidinia yielded a white material giving a true purple colour with Lugol's iodine without any bluish tint, identical, in fact, with that given by the holotrich storage amylopectin. There can be little doubt, therefore, that both epidinia and the holotrichs metabolise glucose in the same way, the only difference being that the latter act very much faster than the former.

Fructose, but not mannose or galactose, also served to keep starved epidinia alive for 24 h, but the increase in storage polysaccharide was not nearly as noticeable as with glucose. With fructose the extracted starch also gave a true purple colour with iodine. Incidentally, the extraction of starch from active holotrich-free cultures of epidinia, whether fed plant starch overnight or not, always yielded material giving a purple colour with iodine with hardly any bluish tint. This proves that the ciliates must metabolise the amylose of plant starch quite rapidly.

Glucosamine as a Substitute for Mannose for Removing Holotrichs from Living Oligotrich Ciliate Suspensions

Sugden and Oxford (1952) found that glucosamine hydrochloride, as well as mannose, was toxic to the rumen holotrich ciliates and that the former acted faster than the latter in immobilising them. It has now been found that glucosamine (0.1–0.5%) is also toxic to certain oligotrich ciliates including *Epidinium* if allowed to act for a sufficiently long time. This toxic action is less evident in the presence of fructose. The following method for removing holotrichs, employing both fructose and glucosamine, worked almost as well as that with mannose (Oxford 1958). The mixed culture of ciliates containing a high proportion of holotrichs, after thorough washing with APB buffer in a 50-ml boiling tube, was treated for $2\frac{1}{2}$ h at 38°c. with 50 ml of the same buffer containing antibiotics and also 0.2% each of glucosamine hydrochloride and fructose (see previous section for effect of fructose on epidinia). The contents of the tube were mixed every 15 min and at the end the bottom deposit was washed by decantation at least 8 times with fresh buffer containing antibiotics and fructose but not glucosamine. The final deposit was placed in a 100-ml conical flask filled with the same buffer containing a little clover starch and green plant material, and incubated overnight, after mixing the contents of the flask on several occasions during the first hour or two. Next morning, the culture consisted of epidinia with normal appearance and sluggish motility and also eudiplodinia which were very active and full of plant material. On two occasions, a very few dasytrichs still survived but their numbers were insignificant by comparison with the oligotrichs. The culture could be maintained for a further day with obvious ingestion of food by epidinia by change of buffer and addition of clover starch. This indicated that the epidinia could recover from whatever damage they might have sustained.

Glucosamine might possibly be employed, at higher concentrations, for freeing living eudiplodinia from both epidinia and holotrichs.

Action of Pluronic Detergents L61 and L62 on Rumen Ciliates in vitro

(a) *L62.* With respect to its action on the oligotrich ciliates, including *Epidinium,* a concentration of 0.05% w/v (about 0.0003M) seemed to be critical. There was a partial cessation of motility in 1 h at this concentration, whereas at 0.1% most died in 1 h and all were dead in 3 h. At a concentration of 0.4%, all were not only dead but also very largely disintegrated in 3 h. Below 0.05%, e.g. at 0.025%, the deleterious action was scarcely noticeable even in 3 h. On the other hand, the holotrich ciliates, at least in the starved state when living on their polysaccharide stores, seemed to be more resistant to the detergent, although there was considerable mortality in 3 h at 0.1% concentration. But even 0.4% did not serve to kill all the isotrichs overnight. The small holotrichs (*Dasytricha ruminantium*) were much more easily killed than the larger isotrichs. The effect of the detergent was much more rapid in the presence of glucose when the holotrichs, having quickly distended themselves with storage polysaccharide formed by metabolism of glucose, were then readily burst by the detergent, even at the 0.1% level, with liberation of clouds of amylopectin granules, exactly as previously observed with the surface active agents Teepol and menthol under the same conditions.

(b) *L61.* Possibly because of the much greater difficulty of dispersing this substance, so that the real concentration of the detergent in the aqueous phase was in all probability lower than expected, its damaging action, particularly on the holotrichs, seemed much less marked than that of L62.

The antibacterial action of these detergents must be very slight since incorporation of 0.3% of L62 into the sloppy lactate agar suitable for growing the anaerobic rumen lactate-decomposing coccus "LC"* (Elsden *et al.* 1956) had not the slightest effect on growth although gas production seemed to be suppressed. The growth of the facultatively anaerobic rumen organism *Streptococcus bovis* is also not affected by L62 (Clarke pers. comm.).

Effect Upon the Rumen Ciliate Population Produced by the Dosing of Cow 294 with Pluronic Detergent L62

Holotrichs and epidinia were the main constituents of the cow 294 rumen micro-fauna before dosing as in spring 1957 (see Part IX). Unless otherwise stated, the following observations refer to rumen contents taken immediately before the morning feed. As far as could be judged by visual inspection, the yield/l of rumen contents represented by the total of the bottom layers of ciliates obtained in the standard separatory funnel method, employing xylose or galactose dissolved in APB buffer, was subject to rapid and considerable natural variation irrespective of the administration of anti-bloat agents to the cow. Thus, with no treatment of the animal intervening, the yields on 16 October and 23 October were obviously much smaller, the latter particularly so, than on 20 October. The infusoricidal effect of L62

* Culture obtained from National Collection of Industrial Bacteria, Teddington, Middlesex.

in vivo would therefore have to be very drastic indeed before it could be concluded with certainty from *in vitro* observations on rumen contents that the detergent had appreciably diminished the rumen ciliate population.

To obtain evidence on this point, an *in vitro* experiment was carried out with rumen contents taken 4 h after feeding, when easiest separation into layers should occur. Addition of L62 to give a final concentration of 0.5%, if evenly dispersed, was found to have a marked effect on the appearance of the rumen contents in that separation into layers did not occur at all. This was due to lack of gas evolution and the falling of a heavy layer of finely divided green material to the bottom. Addition of glucose apparently caused only moderate gas evolution during several hours, without appreciably lifting this heavy green layer. A very minute bottom layer was eventually obtained, microscopic examination of which showed an appreciable proportion of dead and dying ciliates, holotrichs included. It seemed probable, therefore, that if enough L62 was administered to the cow in one dose, and if thorough and rapid mixing did in fact occur in the rumen, the rumen ciliate population ought to be diminished in a short while. A brief examinaton of rumen contents should reveal this fact. This proved to be the case although it was never possible to arrive at an almost complete defaunation of the rumen, even when the detergent was administered daily for several days. The following summarises the *in vivo* experiments carried out with detergent L62:

30 October. 25 ml was administered to cow 294 through fistula just before morning feed. A control was provided by its identical twin No. 293 which received no treatment. The ciliates harvested from the respective rumen liquors before feeding were comparable both in yield and kinds. This was not so 2 h after feeding, when it proved impossible to get a clean separation with 294, although rumen contents from 293 behaved normally. In fact, the 294 rumen contents behaved as in the *in vitro* experiment with L62 just described. At 4 h after feeding, both rumen liquors behaved normally with good separation of ciliate bottom layers in the funnels but the yield given by 293 was obviously much greater in amount than that from 294. Both consisted of the usual mixture of holotrichs and oligotrichs, and in each instance the epidinia had uniformly ingested green plant material.

5 November. 75 ml of detergent L62 administered as before to cow 294; cow 293 received a comparable amount of peanut oil. After 2 h, neither rumen liquor gave a clean separation of ciliates whereas 5 h after feeding both gave very similar but small yields of ciliates without any difficulty. The epidinia in each were very green in appearance. Next morning, after overnight starvation, cow 294 gave a quiescent and non-frothy rumen liquor which yielded far fewer ciliates than the corresponding 0 h control taken 24 h previously. The composition of the mixed culture seemed unchanged save that the smaller isotrichs (*Dasytricha ruminantium*) had been almost eliminated. This result, however, was not confirmed by working up a sample of rumen liquor 4 h later after feeding: both isotrichs and dasytrichs were now in evidence. Next day, before feeding, the yield of ciliates from

294 rumen liquor was quite normal and was many times greater than on the previous day. Holotrichs, mostly isotrichs, seemed now to greatly outnumber oligotrichs.

13–17 November inclusive. 25 ml was administered on each of the first two, and 50 ml on each of the last three days: total 200 ml of L62 in all. Defaunation of the rumen was not achieved, but the ciliate content seemed to be very small on the mornings of 17, 18, and 19 November. The last two mixed cultures consisted chiefly of isotrichs, but some epidinia still remained; dasytrichs were rather hard to find. It was noted also that on the mornings of the 17th and 18th, the rumen liquor was browner in colour than usual and less frothy.

To sum up, copious administration of L62 resulted in an apparent decrease in the rumen protozoan population and also in a qualitative difference in composition in that the large holotrichs tended greatly to predominate over the small holotrichs and also over epidinia. However, 1 week after discontinuance of the L62 dosing, the rumen ciliate population appeared to be perfectly normal again, both quantitatively and qualitatively. The effect of the detergent was merely transient.

DISCUSSION

The fact that epidinia can slowly metabolise glucose is important, not so much on account of their share in the attack on this fodder

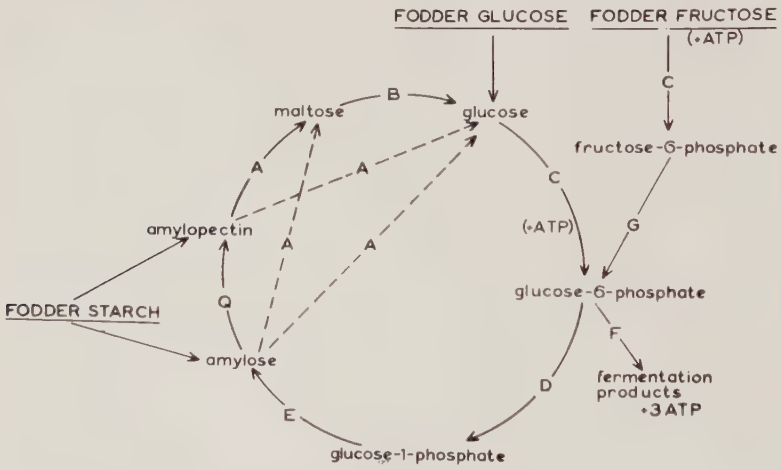


Fig. 1.—*The Rumen Ciliate Starch Cycle.* (i) Full lines, cycle with amylopectin storage; (ii) dotted lines, alternative pathways. Enzymes involved: A. α-amylase (in holotrichs: Howard 1957; Mould and Thomas 1958; in *Epidinium,* Bailey 1958). B. maltase (Howard 1957; Bailey 1958). C. hexokinase. It must be assumed that the epidinial hexokinase, unlike the corresponding holotrich enzyme, has only a weak action on fructose and none at all on mannose. D. phosphoglucomutase. E. starch-synthesising system involving phosphorylase (Mould and Thomas 1958). Q. Q enzyme (Mould and Thomas 1958). F. usual Embden-Meyerhof pathway to fermentation products via triose phosphate. G. phosphoglucoisomerase.

sugar in the rumen, which must be relatively very small in the face of competition by bacteria and holotrich ciliates, but because of the clue thus provided concerning the manner in which both ingested starch grains and their own amylopectin storage grains are dealt with. The following cyclic scheme may be suggested, applying to both epidinia and holotrichs (see Fig. 1):
In this cycle, fodder starch grains, glucose, and fructose are fed in, fermentation products (CO_2, H_2, lactic, acetic, and butyric acids) are excreted, and amylopectin may be temporarily stored as granules inside the organism. The cycle is possible, however, without such storage (see dotted lines).

If availability of ATP determines the extent of starch synthesis, then a *maximum* of two-thirds of the hexose supplied may be stored as amylopectin. The rest must be fermented. Gutierrez's (1955) supposition that the oligotrich ciliates as well as the holotrich ciliates contribute appreciably to VFA production in the rumen is, therefore, in all probability well founded. The extent of this contribution will depend upon the kinds of oligotrich ciliates present in the rumen and their potentialities for amylopectin synthesis and this in turn, no doubt, depends upon the kinds of carbohydrate supplied in the fodder. *Epidinium* has no invertase (Bailey 1958) and is therefore not able to deal with sucrose and levans, but only with starch and to a much lesser extent glucose. Because of this, and other things being equal, it will tend not to be so plentiful, when ryegrass with a low ratio of starch to sugar + levan is fed to the animal, as when red clover with a much higher ratio is fed. This was indeed found to be so in the spring of 1957 (Oxford 1958).

The non-ionic, anti-foam detergent Pluronic L62 may be regarded as almost an ideal agent for inhibiting rumen protozoan action *in vivo* without greatly inhibiting overall rumen fermentative activity, since it seems to have no inhibiting action on typical rumen bacteria. Its infusoricidal action is doubtless due to its appreciable lipid solubility, and it is all the more effective because its water solubility enables it to reach the ciliate endoplasm in much higher concentrations than alcohols, such as menthol, which also act by physical toxicity (cf. Eadie *et al.* 1956). The mode of action of Pluronic L62 in preventing excessive foaming in the rumen may thus be twofold: on the one hand it may act by quickly and effectively breaking foam in the rumen produced by fermentation gases, and on the other hand, by inhibiting protozoan action, it may appreciably diminish gas production in the first few hours after administration. Whether the second effect is ever appreciable in checking bloat, compared with the first, may be doubted. A connection between rumen protozoa and bloat in sheep was long ago suggested by Koffman (1937), who claimed to have demonstrated a change in microfauna with decreased numbers of the larger species in bloating animals. But since a great part of the rumen contents in bloat exists as foam, and since the distribution of large protozoa between the foam and the liquid under it is not uniform, being greater per unit weight in the latter, this difference in microfauna between bloating and non-bloating animals may be more apparent than real if the sample

taken for examination is originally chiefly foam. From the behaviour of rumen contents in the separatory funnel, it seems possible that once stable foam production begins, the relative protozoan activity per unit volume in the liquid part could be greater than would otherwise be the case; in other words, the gas evolution due to protozoa may be accelerated and thus the foaming may, to that extent at least, be progressively worsened. Whether a similar effect can be ascribed to rumen bacteria under foaming conditions is not known.

Acknowledgements

Thanks are due to Syndet (N.Z.) Ltd., Hamilton, for a gift of Pluronic detergents.

References

Bailey, R. W. 1958: Bloat in Cattle. X. The Carbohydrases of the Cattle Rumen Ciliate *Epidinium ecaudatum* Crawley Occurring in Quantity in Cows Fed on Red Clover (*Trifolium pratense* L.). *N.Z. J. agric. Res. 1:* 825-33.

Eadie, J. M.; Mann, S. O.; Oxford, A. E. 1956: A Survey of Physically Active Organic Infusoricidal Compounds and their Soluble Derivatives with Special Reference to their Action on the Rumen Microbial System. *J. gen. Microbiol. 14:* 122.

Elsden, S. R.; Volcani, B. E.; Gilchrist, F. M. C.; Lewis, D. 1956: Properties of a Fatty Acid Forming Organism Isolated from the Rumen of Sheep. *J. Bact. 72:* 681.

Gutierrez, J. 1955: Experiments on the Culture and Physiology of Holotrichs from the Bovine Rumen. *Biochem. J. 60:* 516.

Heald, P. J. 1952: The Fermentation of Pentoses and Uronic Acids by Bacteria from the Rumen Contents of Sheep. *Ibid. 50:* 503.

Howard, B. H. 1957: Biochemical Studies of Individual Genera of Rumen Holotrich Protozoa. *Ibid. 67:* 18P.

Koffman, M. 1937: Connection between Protozoa of the Rumen and Tympany (Translated title). *Skand. VetTidsskr. 27:* 45; *Vet. Bull. Weybridge, 7:* 592.

Masson, F. M.; Oxford, A. E. 1951: The Action of Ciliates of the Sheep's Rumen upon Various Water Soluble Carbohydrates Including Polysaccharides. *J. gen. Microbiol. 5:* 664.

Mould, D. L.; Thomas, G. J. 1958: Degradation of Starch by Holotrich Protozoa from Sheep Rumen. *Biochem. J. 69:* 327.

Oxford, A. E. 1951: The Conversion of Certain Soluble Sugars to a Glucosan by Holotrich Ciliates in the Rumen of Sheep. *J. gen. Microbiol. 5:* 83.

———1958: Bloat in Cattle. IX. Some Observations on the Culture of the Cattle Rumen Ciliate *Epidinium ecaudatum* Crawley Occurring in Quantity in Cows Fed on Red Clover (*Trifolium pratense* L.) *N.Z. J. agric. Res. 1:* 809-24.

Pucher, G. W.; Leavenworth, C. S.; Vickery, H. B. 1948: Determination of Starch in Plant Tissues. *Anal. Chem. 20:* 850.

Sugden, B.; Oxford, A. E. 1952: Some Cultural Studies with Holotrich Ciliate Protozoa of the Sheep's Rumen. *J. gen. Microbiol. 7:* 145.

STICKY TRAPS FOR WINGED APHIDS

By R. Close, Crop Research Division, Department of Scientific and Industrial Research, Lincoln

(*Received for publication, 11 December 1958*)

Summary

Modifications of the sticky cylinder technique for trapping winged aphids are described. Cup grease was found to be very satisfactory for trapping aphids, and the aphids could be removed from the traps in the field. The advantages of these changes are discussed and the results of a season's trapping presented.

Introduction

The basis for any study of aphids as virus vectors is the adoption of a suitable technique for trapping the winged forms. For trapping in widely separated areas it is desirable to use traps which require the minimum of attention and can be serviced easily. Moericke water traps used in preliminary trials at Lincoln needed very frequent attention and suction traps need a source of power; neither of these methods is therefore suitable. Watts (1953) and Hollings (1955) have found that yellow cylindrical sticky traps (Broadbent 1948) are suitable for surveys of populations of winged aphids. This method was adopted, and modified. In this paper these modifications are described and preliminary results from the trapping of aphids during a summer season, November 1957 to May 1958 are presented.

Methods

The Trap

Galvanised-iron stove pipes, five inches in diameter and one foot in length were screwed to a light stake. These stove pipes had been given several coats of a zinc chromate primer which Dr. K. P. Lamb (pers. comm.) had found attracted more aphids than other types of yellow paint. Cellulose acetate film, 10/1000 inch thick, was placed around the outside of the trap and held in place by a 3/16 inch brass rod (Fig. 1). The film was cut to fit round the bracket at the base. A cup grease with a consistency of 355–385 at 77°F., as measured by the ASTM penetrometer, was used as a thin layer 1 to 2 mm thick applied to the film with a paint brush. The efficiency of this grease in trapping aphids was compared at Lincoln with a tree-banding grease, 'Stictite' (Hollings 1955) and with a spray-pump grease.

Traps were erected with the base of the stove pipe 26 in. from the ground and with the brass rod facing north-west so that an indication of the direction of aphid flight could be obtained. The traps were inspected weekly in the field. All insects that appeared to be aphids were removed from each trap with a knife and transferred to a glass slide, a small amount of grease being scraped off with the insects to minimise damage. The grease and other insects were then scraped off the film and a fresh layer of grease applied, the film remaining in place on the trap.

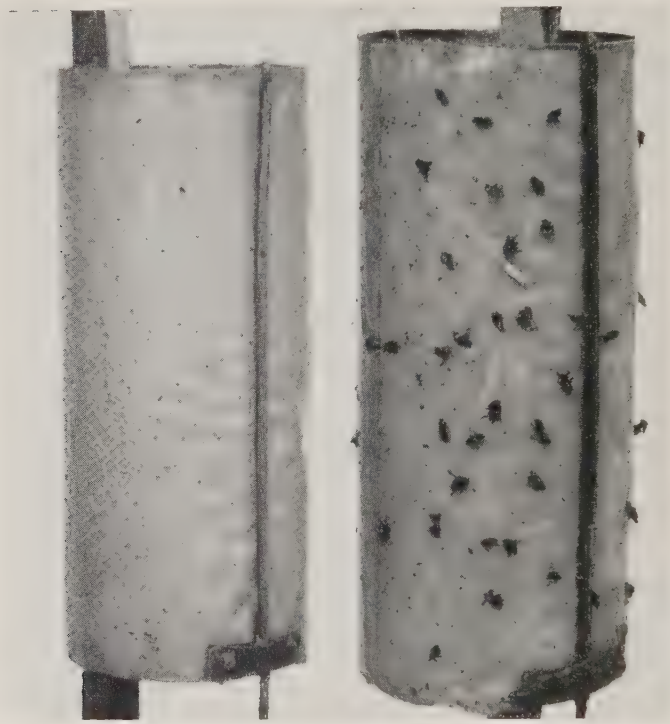


Fig. 1.—Cylindrical sticky traps with (*left*) cup grease with aphids and other small insects, (*right*) tree-banding grease with aphids, other small insects, and large flies.

Photo: *C. J. Miles*

Clearing Aphids of Grease

The glass slide carrying the aphids was placed in a sample tube and covered with a mixture of equal parts of ethyl acetate and acetic acid. Aphid samples could be stored in this way and a number cleared at the one time. With few aphids the grease would dissolve overnight but if there were many aphids then the tubes were placed in a water-bath at 80–90°C. for 10 minutes. The glass slide, free of aphids, was then removed and the liquid drawn off through capillary tubing into a Buchner flask. The process was repeated until the aphids were free from grease. After the final clearing the aphids were washed with a mixture of equal parts of 50/50 ethyl acetate—acetic acid and 70% alcohol and then with 70% alcohol. All these operations were carried out in a fume cupboard. The sample was then transferred to a petri dish and sorted under a low-power lens (×2), the aphids being counted into fresh 70% alcohol and all other insects discarded. Other workers have stored the aphids in phenol but for large numbers of aphids it was found more convenient to sort and store samples in alcohol and to do preliminary identifications.

Sites

Traps were placed at the following three sites in Canterbury:—Lincoln (36 ft above sea level, two traps, 10 feet apart to obtain comparisons between greases), Darfield (600 ft above sea level) and Annat (1,100 ft above sea level). The traps were either within or adjacent to a potato crop.

RESULTS

Aphids could be readily located on the traps and collected although when there were large numbers on the trap it is possible that a very few were missed. The time taken to change a trap was from 10 to 20 minutes, depending on the number of insects caught. The cup grease was easy to apply and remove and proved very satisfactory for trapping aphids. It seemed unaffected by high temperatures but was indented by heavy rain; the effect of rain on the trapped aphids appeared negligible.

In Table 1 the weekly totals of aphids caught at each of the sites are presented. Information on the types of aphids caught will be presented subsequently.

TABLE 1. WEEKLY TOTALS OF WINGED APHIDS COLLECTED FROM SINGLE TRAPS AT THREE LOCATIONS

Week Ending	Lincoln	Darfield	Annat
8/11/57	23	20	—
14/11/57	187	12	33
22/11/57	283	16	52
29/11/57	219	19	18
6/12/57	155	23	25
13/12/57	50	17	41
20/12/57	74	12	20
27/12/57	17	5	4
3/ 1/58	8	2	8
10/ 1/58	10	7	0
17/ 1/58	24	1	2
24/ 1/58	11	2	0
31/ 1/58	41	10	7
7/ 2/58	24	6	63
14/ 2/58	49	20	35
21/ 2/58	44	10	56
28/ 2/58	220	9	164
7/ 3/58	940	189	253
14/ 3/58	1188	15	68
21/ 3/58	123	39	80
28/ 3/58	50	0	41
3/ 4/58	115	2	19
10/ 4/58	12	—	—
17/ 4/58	18	—	—
24/ 4/58	22	—	—
1/ 5/58	4	—	—

From the table it can be seen that the main aphid flights occurred at approximately the same time in each district. Fewer aphids were

caught at Darfield and Annat than at Lincoln and this is to be expected, because these areas are at a greater altitude.

The results of a comparison of the three greases at Lincoln are given in Table 2. These are the results from one trap per grease, expressed as weekly totals of winged aphids.

TABLE 2. COMPARISON OF THREE GREASES, ONE TRAP PER GREASE, WEEKLY TOTALS OF WINGED APHIDS

Week Ending	Cup Grease	Spray-pump Grease	
14/2/58	49	47	
21/2/58	44	45	
28/2/58	220	144	
7/3/58	940	1165	
	1253	1401	
	Cup Grease	"Stictite" Grease	Large Flies on "Stictite"*
14/3/58	1188	958	185
21/3/58	123	93	155
28/3/58	50	44	52
3/4/58	115	85	28
	1476	1180	420

* No large flies were caught on the cup and spray-pump greases.

Although there were no substantial differences between greases, there is a suggestion that the cup grease is more effective than the 'Stictite' grease. This may be because many large flies were caught on the 'Stictite' grease, reducing the area available to aphids (Fig. 1). The spray-pump and 'Stictite' greases are relatively viscous, difficult to apply and remove, and hence not as suitable for the traps.

As already stated, the aphids can be cleared of grease by the use of 50/50 ethyl acetate-acetic acid, are relatively undamaged and can be identified in the alcohol. If necessary they can be transferred to a gum-chloral mount for further examination.

Large numbers of other small insects are caught on the traps. These are primarily small Diptera (midges, fungus gnats, and flies), though small wasps, beetles, weevils, and spiders are also trapped. The Argentine stem weevil (*Hyperodes griseus* Hust) and *Aphodius granarius* Linn. have been found on several occasions, while staphylinid (*Conosomus* sp. and *Trogophloeus* sp.) and lathridiid (*Micrambina* sp.) beetles are invariably present. Shield and capsid bugs are sometimes trapped, but most moths, bees, and other large insects are able to escape from the cup grease, though many were caught on the 'Stictite' grease.

DISCUSSION

Since Broadbent (1948) showed that the colour of sticky traps for winged aphids should be yellow instead of white there have been few modifications of the method. The chief advantage of the method here presented is its ease of operation, for although it is similar to the original method there is no handling of the sticky sheets. This has been made possible by the use of cup grease which is of a consistency that enables the trap to be serviced in the field.

The cylindrical trap with cup grease has proved very satisfactory for the trapping of winged aphids in Canterbury and could be adopted for general surveys of aphid populations throughout New Zealand. This coming season it is planned to maintain three traps at each of the three sites, and so obtain a clearer understanding of aphid movements, in relation to the virus diseases of potatoes and brassicas.

ACKNOWLEDGEMENTS

The writer wishes to acknowledge the technical assistance of Mrs. D. T. P. Gallagher and Mr. H. W. Findon of the Crop Research Division, the co-operation of Mr. F. W. Jarman, Darfield, and Mr. G. E. Wright, Annat, on whose properties the traps were located and who attended to them weekly; and the assistance of Miss E. Stevenson, Applied Mathematics Laboratory for statistical analyses; Mr. L. J. Dumbleton and Mr. E. S. Gourlay of the Entomology Division for the identification of insects and officers of the Shell Company of New Zealand Ltd. for assistance with the selection of greases.

REFERENCES

Broadbent, L. 1948: Aphis Migration and the Efficiency of the Trapping Method. *Ann. appl. Biol. 35:* 379-94.

Hollings, M. 1955: Aphid Movement and Virus Spread in Seed Potato Areas of England and Wales, 1950-1953. *Plant Path. 4:* 73-82.

Watts, B. B. 1953: The Aphids of the Potato Crop. *M.Ag.Sc. Thesis,* Canterbury Agricultural College.

NEW RECORDS OF FUNGOUS DISEASES IN NEW ZEALAND, 1957-58

By Joan M. Dingley, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(*Received for publication, 22 September 1958*)

Summary

The following fungi are recorded from New Zealand for the first time:—*Graphiola phoenicis* (Moug.) Poit., *Septoria passiflorae* Louw, *Septoria petroselini* Desm., *Colletotrichum graminicola* (Ces.) Wilson, *Pleiochaeta setosa* (Kirchner) Hughes, *Ramularia centranthi* Brun., *R. deusta* (Fckl.) Baker, Snyder, et Davis, *R. eximia* Bubak, *R. picridis* Fautr. et Roum., *R. rhabdospora* (Berk. et Br.) Nannf., *R. rubella* (Bon.) Nannf., *R. taraxaci* Karsten.

Additional host records include *Albugo tragopogonis* Pers. ex Gray, on cineraria (*Senecio cruenta* DC), *Sphaerotheca fuliginea* (Schlecht.) Poll. on *Cucurbita pepo* L., and *Alternaria citri* Pierce on leaves of citronelle (*Citrus* sp.).

The following records are based on material collected during the past twelve months. In all cases specimens have been placed in the herbarium of the Plant Diseases Division, Auckland, the herbarium identification number being placed in brackets after the collection.

1. *Graphiola phoenicis* (Moug.) Poit., Ann. Sci. nat. *3:* 473; 1824.
On *Phoenix canariensis* Chab.
Kerikeri Central, North Auckland, Dec. 1957, R. Saxton (18212).

Both surfaces of diseased leaves are spotted with black erumpent pustules 1–2 mm in diameter, 0.5–1 mm high. These erumpent pustules are surrounded by a black sterile outer wall. Within this carbonaceous outer wall small clusters of dark, upright, septate hyphae are developed among which yellow, powdery, sporogenous tissues are formed. Spores are unicellular, more or less globose and lightly coloured, 2.5–4.5 μ diameter. This fungus occurs in most countries where palms are grown. Although not typical of most smut fungi, it is usually classified in the Ustilaginales. It has been collected in the one locality only in New Zealand.

2. *Septoria passiflorae* Louw, S. Afr. Dep. Agric. Sci. Bull. *229:* 34; 1941.
On *Passiflora edulis* Sims.
Epsom, Auckland, March 1958, J. M. Dingley (18287) (Fig. 1).

Many specimens of diseased fruit and leaves were collected in the vicinity of Auckland city during autumn, 1958. Small, light-brown lesions 0.5–1 cm diameter appeared on leaves and surfaces of fruits; pycnidia appear as small, dark-coloured pinspots on the disease lesions. They are usually subepidermal and globose, and vary from 50–150 μ, thin-walled, and with a distinct ostiole. Pycnospores are hyaline, filiform 1–3 septate, 12–25 $\times$ 1–1.5 μ.

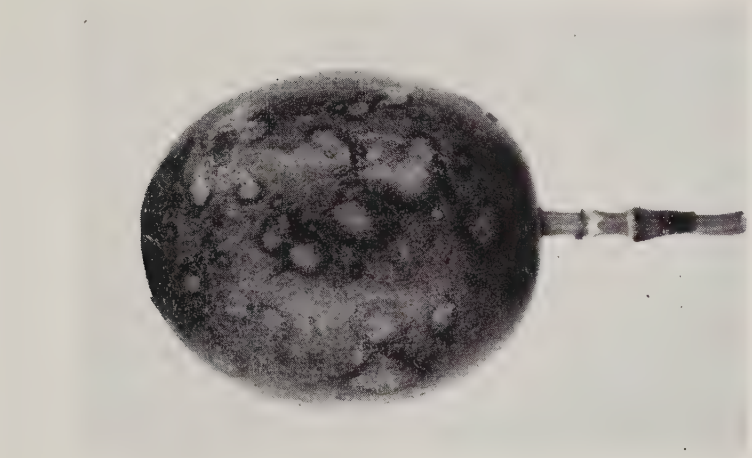


Fig. 1.—Passion fruit infected with *Septoria passiflorae*.
Photo: J. W. Endt

The disease lesions never show the outer, reddish-brown ring typical of those formed by *Alternaria passiflorae* Simmonds; they may be distinguished from grease spot (*Pseudomonas passiflorae* W. D. Reid) as examination of lesions with a hand lens usually shows the presence of pycnidia. *Septoria passiflorae* has been reported from Queensland as well as South Africa.

3. *Septoria petroselini* Desm., in Kickx, "Flore Cryptogamique des Flandres," Vol. 1, p. 424: 1867.
On leaves of *Petroselinum crispum* (Mill.) Nym.
Mt. Albert, Auckland, March 1953, S. D. Brook; Henderson, Auckland, April 1958 (18328); Christchurch, Canterbury, March 1958, A. Mill (18260).

The fungus forms pale-coloured lesions, brown at the margins, 1–3 mm diameter, often covering a whole segment of the leaf. Lesions are dotted with minute, black pycnidia 60–100 μ diameter; Pycnospores are hyaline, filiform, straight, 1–3 septate, 30–50 $\times$ 1–1.5 μ.

The fungus has been present in New Zealand for a number of years. Collections in the herbarium date back to 1921. It is common throughout the world wherever parsley is grown.

4. *Colletotrichum graminicola* (Ces.) Wilson, Phytopathology *4:* 110; 1914.
On *Bromus catharticus* Vahl.
Mt. Albert, Auckland, Aug. 1956, J. M. Dingley (16368).
On *Holcus mollis* L.
Kimball, Otago, Feb. 1957, A. J. Healy (17557).
On *Lolium perenne* L.
Mt. Albert, Auckland, Aug. 1958, J. M. Dingley (18461).
On *Paspalum dilatatum* Poir.
Mt. Albert, Auckland, May 1956, J. M. Dingley (18320).

In most warmer countries 'anthracnose' has a wide host range on cereals and grasses causing both a seedling blight and a leaf spot. In New Zealand up to the present it has only been found on four species and is associated in all cases with leaf yellowing. Fructifications of the fungus occur on necrotic areas on both surfaces of the culm; they form dark, elliptical hairy acervuli 0.5–1 mm long, often more or less fusing together; setae surrounding the acervulus are 100–120 μ long, single-celled, sometimes with one or more cross septa; conidia are borne on unbranched conidiophores in the erumpent acervuli: they are hyaline, falcate, unicellular, 17–30 $\times$ 3–5 μ.

Von Arx (1957) states that this fungus is the same organism as *Glomerella tucumanensis* (Speg.) V. Arx et Muller, described from sugar cane and sorghum; no perithecia have been found in New Zealand.

5. *Pleiochaeta setosa* (Kirchner) Hughes, Commonwealth Mycological Institute, Mycol. Pap. *36:* 39; 1951.
On leaves of seedling *Lupinus angustifolius* L.
Mt. Albert, Auckland, July 1958, J. Robinson (18401) (Fig. 2).

Fig. 2.—Lupin leaf showing disease lesions of *Pleiochaeta setosa.*
Photo: S. A. Rumsey

Lesions at first minute, purplish-brown dots, rapidly increasing in size to 2–3 mm diameter, zoned, the outer ones being light-greyish and translucent. Thick, lightly pigmented mycelium 4–10 μ diameter is formed immediately below the cuticle. Conidiophores

are produced superficially on both surfaces of the leaf; they are usually formed singly, and are up to 80 μ long. Conidia are formed terminally, a new growing point being formed immediately below the scar of the last-formed conidium. Conidia 65–88 × 12–17.5 μ, fusiform, 4–6 septate, terminal cell forming 1–4 setae, the terminal appendage sometimes branching; terminal and basal cell walls hyaline, others lightly pigmented (Fig. 3). The fungus has been recorded overseas on lupins (*Lupinus* spp.), brooms (*Cytisus* spp.), and *Crotolaria* sp.; it infects leaves, stems, and pods causing defoliation and sometimes die-back.

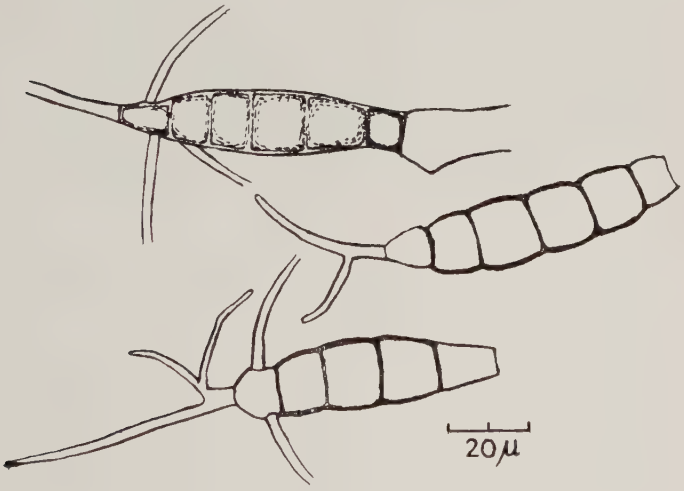


Fig. 3.—Conidia of *Pleiochaeta setosa*.

6. *Ramularia* spp.
Ramularia centranthi Brun., J. d'hist. nat. de Bordeaux et du Sud Ouest, p. 430; 1887.
On *Kentranthus ruber* (L) DC.
Mt. Albert, Auckland, Feb. 1958, J. Robinson (18252).
Ramularia deusta (Fckl.) Baker, Snyder, et Davis, Mycologia *42:* 416; 1950.
On *Lathyrus latifolius* L.
Palmerston North, Wellington, Jan. 1924, J. C. Neill (1593); Upper Hutt, Wellington, April 1953, A. J. Healy (12152); Campbells Bay, Auckland, July 1953, J. M. Dingley (12338).
On *Lathyrus pratensis* L.
Ross, Westland, Feb. 1947, A. J. Healy (12172).
Ramularia eximia Bubak, S. B. böhn. Ges. Wiss. 18; 1903.
On *Crepis* sp.
Mt. Albert, Auckland, Nov. 1951, J. M. Dingley (10965).
On *Crepis capillaris,* L.
Mt. Albert, Auckland, Nov. 1956, J. M. Dingley (17164).
Ramularia picridis Fautr. et Roum., Rev. Myc. *13:* 133; 1891.
On *Picris echioides* L.

Palmerston North, Wellington, Jan. 1924, J. C. Neill (1601); Petone, Wellington, Nov. 1953, A. J. Healy (12861); Christchurch, Canterbury, Jan. 1955, A. J. Healy (14282); Mt. Albert, Auckland, Nov. 1956, J. M. Dingley (18229).

Ramularia rhabdospora (Berk. et Br.) Nannf., Lundell and Nannfeldt, Fungi Exsic. Suec. No. 1989; 1950.

On *Plantago lanceolata* L.

Haywards, Hutt R., Wellington, May 1953, A. J. Healy (12173): Half Moon Bay, Stewart I., Feb. 1956, J. M. Dingley (16098).

Ramularia rubella (Bon.) Nannf., Lundell and Nannfeldt, Fungi Exsic. Suec., No. 1992; 1950.

On *Rumex obtusifolius* L.

Featherston, Wellington, March 1953, A. J. Healy (12119); Appleby, Nelson, April 1956, S. D. Brook (16013); Christchurch, Canterbury, Jan. 1956, A. M. Thompson (16081); Mt. Albert, Auckland, Aug. 1956, J. M. Dingley (16371).

On *Rumex crispus* L.

Weraroa, Wellington, Sept. 1919, G. H. Cunningham (63).

Ramularia taraxaci Karsten, Hedwigia *23:* 7; 1884.

On *Taraxacum officinale* L.

Wellington city, Oct. 1953, A. J. Healy (12687); Upper Hutt, Wellington, Jan. 1953, A. J. Healy (11753); Riccarton, Canterbury, Feb. 1953, A. J. Healy (14262); Temuka, Canterbury, Dec. 1956, A. J. Healy (17558).

In *Ramularia* the different species are host specific, attacking at the most only a small number of closely related host plants; for example Lundell and Nannfeldt (1950) in listing *Ramularia* occurring on different *Lathyrus* spp. note that a different species, *R. alba* (Dows) Nannfeldt, attacks sweet peas (*Lathyrus odoratus* L.) and is distinct from *R. deusta* which is recorded here on *L. latifolius* and *L. pratensis.*

These fungi form more or less definite buff-coloured lesions on leaves, stems, and petioles. Minute clusters of conidiophores occur on the undersurfaces of leaves; conidia are hyaline or lightly coloured, variable in size and shape, oval or cylindrical but with tapering ends, sometimes unicellular, but often with one or more transverse septa. They are borne singly at ends of conidiophores but the single conidia may themselves catenulate conidia. Because of the variation in conidia these fungi have been described under a number of genera in the Moniliales (especially *Ovularia* and *Cercosporella*).

Additional Host Records

7. *Albugo tragopogonis* Pers., ex S. F. Gray, "A Natural Arrangement of British Plants", Vol. 2, p. 540; 1821.

On *Senecio cruentus* DC.

Lower Hutt, Wellington, Jan. 1958, S. P. Money (Fig. 4).

White pustules of conidia were formed on undersurfaces of leaves of young cineraria plants. Infected parts of the young

Fig. 4.—Conidial pustules of white rust (*Albugo tragopogonis*) on cineraria leaves.
Photo: S. A. Rumsey

leaves collapse to form sections of dead tissues.
Albugo tragopogonis is recorded in New Zealand by Baker (1955) on a number of host plants belonging to Compositae.

8. *Sphaerotheca fuliginea* (Schlecht.) Poll., Atti Ist. bot. Univ. Pavia, ser. 2, *9:* 158; 1911.
syn. *Sphaerotheca humuli* var. *fuliginea* (Schlecht.) Salmon.
On *Cucurbita pepo* L.
Henderson, Auckland, June 1958, S. D. Brook (18372).

Perithecia of this fungus were previously described from New Zealand on *Coriaria* sp. (Dingley and Brien 1956). In this present collection on leaves of an ornamental gourd the perithecia are larger (85–105 μ diam.) and asci often contain only 6 ascospores; ascospores are 12–16 × 10–14 μ. Leaves of the gourd were covered with a feltlike mat of conidiophores of the oidium stage among which perithecia are formed. Superficially the diseased leaves may be distinguished from those infected with *Erysiphe cichoracearum* DC by the pale brownish colour of the conidial mass. Homma (1937) notes that mature conidia of this species of powdery mildew contain well developed 'fibrosin' bodies not found to such a marked extent in conidia of *E. cichoracearum*.

9. *Alternaria citri* Pierce, Bot. Gaz. *33:* 234: 1902.
On leaves of *Citrus* sp. (citronelle).
Mt. Albert, Auckland, Feb. 1958, P. J. Brook (18251).

In New Zealand this fungus is recorded as a fruit rot on New Zealand grape fruit (Cunningham 1931) and on sweet orange (Brien 1939). In this present record the fungus is associated with

a leaf spot which is responsible for a considerable amount of defoliation; fructifications of the fungus occurred on both upper and lower surfaces of the leaves.

References

Baker, Shirley D. 1955: *Trans. roy. Soc. N.Z. 82:* 987-93.

Brien, R. M. 1939: *N.Z. Dep. sci. industr. Res. Bull. 67:* 39 pp.

Cunningham, G. H. 1931: *N.Z. J. Agric. 42:* 305-6.

Dingley, Joan M.; Brien, R. M. 1956: *N.Z. J. Sci. Tech. A38:* 434-9.

Homma, Usa 1937: *J. Fac. Agric. Hokkaido Univ. 38:* 186-461.

Lundell, Seth; Nannfeldt, J. A. 1950: "Fungi Exsiccati Suecici," Fasc. 39-40.

Von Arx, J. A. 1957: *Phytopath. Z. 29:* 413-68.

CONCENTRATIONS OF END-PRODUCTS AND MORPHOLOGICAL TYPES OF RUMEN BACTERIA IN SILAGE-FED SHEEP

By V. J. Williams* and K. R. Christian†, Ruakura Animal Research Station, Hamilton

(*Received for publication, 9 September 1958*)

Summary

Rumen fermentation curves were obtained for sheep fed four silages of different quality. Peak levels of volatile fatty acids (VFA) and ammonia were reached within one hour of the start of feeding. Protein nitrogen (PN) levels fell after feeding as in grass-fed sheep.

In another series of trials, rumen samples were taken two hours after feeding from ten groups of sheep fed ten silages of different compositions. Ruminal pH and VFA levels were constant irrespective of silage pH, VFA, and lactic acid content. Ruminal ammonia levels were related more to silage ammonia and residual nitrogen (RN) levels combined than to either level separately. Ruminal PN levels were low and RN levels high on all silages.

It is suggested that levels of rumen end-products are dependent more on the combined soluble constituents, bacterial products plus grass solubles, than on individual constituents or digestibility of the silage.

Introduction

It is usually regarded as an essential part of the ensiling process that at least part of the soluble contents of the plant cells is released for bacterial fermentation as the result of anoxia and mechanical rupture of the cell walls. The extent and nature of the fermentation is profoundly modified by the method of silage making, involving varying degrees of wilting, compaction, and laceration, and sometimes the use of chemical additives. Such conditions as temperature and oxygen tension may be varied greatly by these means. In all cases, however, plant carbohydrates are partly converted to lactic or volatile acids and protein material is degraded to ammonia and other simple nitrogenous compounds.

This process is somewhat similar to that taking place within the rumen. Silage may, therefore, be considered as plant material that has already been partly digested, and the normal end-products of rumen digestion are released into the rumen without the action of rumen micro-organisms.

The only published work on rumen metabolism of animals fed silage appears to have been made by Elliot *et al.* (1957), who compared the feeding of corn silage and hay-crop silage as the only sources of roughage. Corn silage, which was higher in acetic and lactic acids, but lower in butyric acid, protein, and fibre content than hay-crop silage, gave higher ruminal total volatile fatty acids and a higher proportion of propionic acid in the rumen.

* Now University of New England, Armidale, New South Wales.
† Now Mt. Pleasant Laboratories, Dept. of Agriculture, Launceston, Tasmania.

Since silage is a multi-purpose supplement of great economic importance in pasture conservation, a study of the rumen fermentation of silages of different composition promised to be of interest. Work has, therefore, been carried out on sheep used by Mr. R. J. Lancaster for silage digestibility studies.

EXPERIMENTAL

Fermentation Curves

Four silages of different quality were fed to groups of 3 sheep over three-weekly periods. On at least 2 days during the last fortnight of the trials, the animals were rumen-sampled at hourly intervals before and after the morning feed, which constituted half the daily ration, and which was eaten completely within one hour. The previous day's ration had been fed 24 hours before and had been fully consumed within 2 hours.

Water was not available during the sampling periods.

Single Samplings

Groups of 3 sheep were fed ten different silages over three-weekly periods, and rumen samples were taken on 5 or more days during the last fortnight of each trial 2 hours after being given their daily feed, which was largely or completely eaten within this time.

Methods

Analysis of the strained rumen fluid for pH, volatile fatty acids (VFA), ammonium nitrogen, protein nitrogen (PN) and residual

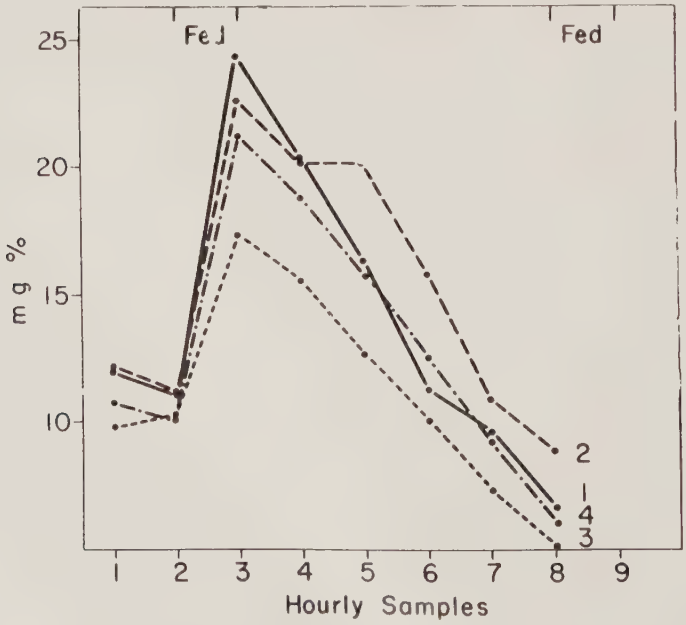


Fig. 1.—Ammonium nitrogen (mg %).

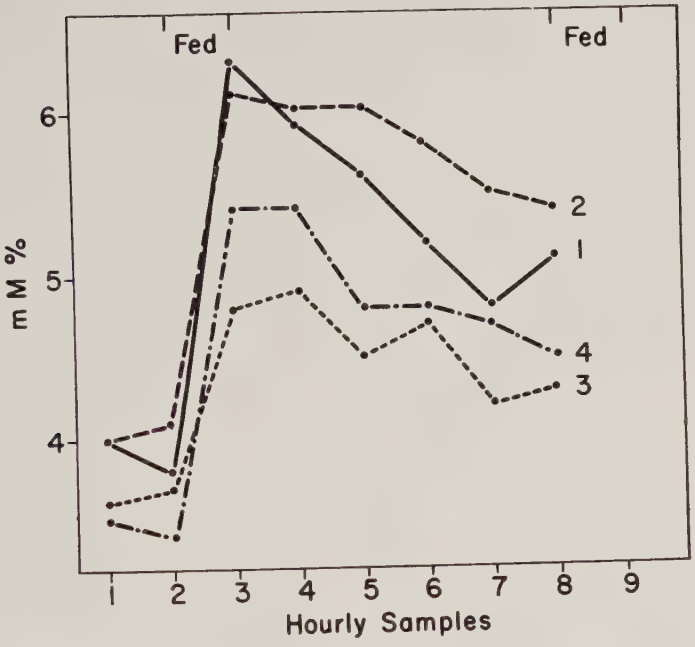


Fig. 2.—Volatile fatty acids (mM %)

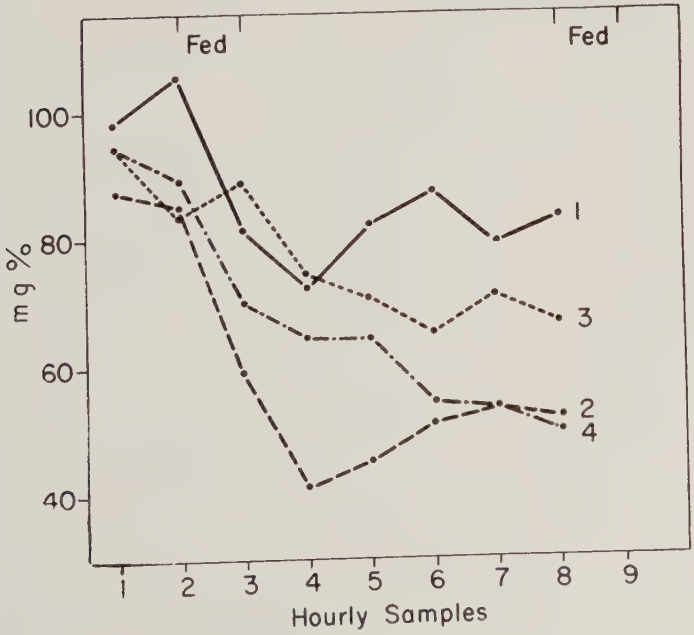


Fig. 3.—Protein nitrogen (mg %).

nitrogen (RN), were carried out as described earlier (Christian and Williams 1957). Counts of total numbers and of morphological types of "free" bacteria, using a negative nigrosine stain, were made for the single-sampling series.

Results

Analysis of silages 1–4 and of silages 5–14 (on a dry-matter percentage basis) for which the fermentation-curves series and the single-samplings series respectively were obtained, are presented in Table 1. Constituents common to both silage and rumen fluid are compared on a wet-matter basis in Table 2. Weight percentages of volatile fatty acids are calculated as acetic acid, and reducing sugars as glucose. Table 3 summarises the bacteriological findings for silages 5–14.

Fermentation curves for silages 1–4 are shown in Figs. 1–3.

TABLE 1. Analysis of Silages
(D.M. basis)

Silage No.	D.M. (%)	*O.M. Dig. %	pH	VFA (g%)	Lactic Acid (g%)	Sugars (g%)	Ammon. N. (g%)	PN (g%)	RN (g%)	Total N. (g%)
1	18.4	56	4.8	3.21	3.41	—	0.60	—	—	2.80
2	15.0	66	4.4	1.45	5.34	—	0.36	—	—	2.53
3	12.8	58	5.2	4.55	0.25	—	0.80	—	—	2.58
4	14.8	64	4.2	4.81	5.12	—	0.30	—	—	2.46
5	18.5	70	4.6	4.01	2.52	2.6	0.71	2.30	1.40	
6	17.6	73	4.4	2.57	7.02	2.0	0.35	2.10	1.40	
7	19.1	75	4.6	1.54	4.50	7.7	0.34	1.70	1.70	
8	16.1	69	5.3	6.33	0.54	2.6	1.19	1.40	1.20	
9	17.2	74	4.6	2.57	6.03	5.1	0.54	1.50	1.70	
10	18.6	68	4.0	2.98	9.10	2.1	0.33	1.80	1.40	
11	23.8	56	4.0	1.27	6.48	5.2	0.17	1.00	0.51	
12	24.0	56	4.0	1.18	4.32	8.0	0.14	1.00	0.58	
13	14.9		5.6	12.20	0.09	—	1.29	1.70	0.51	
14	15.3		5.5	10.46	0.90	—	1.15	1.45	0.87	

* Oganic matter digestibility per cent.

Discussion

Intakes of silage dry matter could not be completely standardised in these trials, and it has been found (unpubl. data) that level of intake of fresh grass may have some effect on peak concentrations of the fermentation curves. However, the differences to be expected from this source are unimportant compared with the wide range in composition of silage fed. The disparity in intakes between silages 1–4 and silages 5–14 is too great to allow direct comparisons to be made between these two groups.

Peak values for ammonia (Fig. 1) and VFA (Fig. 2) occurred somewhat earlier than when fresh grass is fed (Christian and Williams

TABLE 2. RUMEN AND SILAGE ANALYSES
(2 hours after feeding)

Silage No.	Intake Fresh Matter /Feed	Dry Matter %	Intake Dry Matter /Feed	Constituents on fresh Matter Basis													
				pH		VFA mM%		Amm. N mg%		RN mg%		PN mg%		Lactic Ac. mM % Silage	Sugars mg% Silage	NH_3 + RN mg%	*VFA Potential of Silage mM%
				Sil.	Rumen	Sil.	Rumen	Sil.	Rumen	Sil.	Rumen	Sil.	Rumen				
1	800	18.4	147	4.8	—	11.4	5.9	110	20.3	—	—	—	72	7.0	—		
2	1000	15.0	150	4.4	–	4.2	6.0	54	20.0	—	—	—	41	8.9	—		
3	1070	12.8	137	5.2	—	11.3	4.9	102	15.5	—	—	—	74	0.4	—		
4	1020	14.8	151	4.2	—	8.9	5.4	44	18.6	—	—	—	64	8.5	—		
5	2640	18.5	490	4.6	7.6	14.4	8.0	131	32.5	260	58	425	52	5.2	480	391	24.9
6	2880	17.6	506	4.4	7.5	8.8	8.0	61	26.4	245	46	368	66	13.6	350	306	26.3
7	3000	19.1	570	4.6	7.5	5.7	8.0	65	24.4	325	79	325	47	9.6	1470	390	31.6
8	2440	16.1	394	5.3	7.6	19.8	6.7	192	21.8	194	21	226	52	1.0	420	386	25.5
9	2720	17.2	468	4.6	7.4	8.6	7.9	93	26.7	293	39	258	50	11.5	880	386	29.9
10	2420	18.6	450	4.0	7.6	10.8	7.6	61	21.8	260	25	335	51	18.8	390	321	33.9
11	2320	23.8	554	4.0	7.4	6.7	8.4	40	12.9	121	20	238	52	17.1	240	161	26.5
12	2300	24.0	554	4.0	7.2	5.5	8.7	34	13.2	139	16	240	46	11.5	1920	173	38.5
13		14.9		5.6	7.4	35.3	8.0	192	24.8	76	21	253	55	0.1	—	268	—
14		15.3		5.5	7.6	31.0	7.8	177	24.5	133	28	222	42	1.5	—	310	—

* VFA + lactic acid + sugars

TABLE 3. RUMEN ORGANISMS (SINGLE-SAMPLING SERIES)

Silage No.	Total Free Count (10^6/cu. mm.)	Single cocci	*Morphological types %		
			Diplo-cocci	Curved Rods	Straight Rods
5	15	53	8	6	33
6	18	58	6	7	29
7	16	59	6	5	30
8	18	60	11	4	25
9	16	63	8	4	25
10	19	57	6	4	32
11	22	59	8	4	28
12	19	58	10	4	28
13	23	61	5	5	29
14	21	64	5	5	26
Dried grass	35	59	4	5	32

* 1% chains of cocci in silage No. 11. No spirilla types in any sample

1957), which would appear to be due to the extensive physical breakdown of plant structure and pre-fermentation with the consequent rapid release of a large proportion of the soluble constituents into the rumen. The fall in PN after feeding (Fig. 3) is similar to that found in grass-fed animals. The shapes of the curves are not greatly different for the different silages, and samples taken 2 hours after feeding, therefore, provide a reasonable indication of peak values.

If silage of known composition enters a rumen of given volume the change in concentration of any constituent may be calculated theoretically, subject to reservations, for instance the admission of 1210 g of silage with a lactic acid content of 18.8 mM per cent (silage 10) would, if immediately released into a rumen containing 4 litres of fluid, produce a concentration of 4.4 mM per cent. Briggs *et al* (1957) have found that, in wheat feeding, levels higher than 2.0 mM per cent lactic acid produced a rumen pH of less than 5.0, and similar findings have been obtained at this Station after the feeding of sucrose or fodder beet (unpubl. data). However, silage lactic acid is seen to have had no effect on rumen pH levels (Table 2), which were consistently higher than in grass-fed animals. Dosing 130 mM/day of lactic acid into the rumen of sheep has been found to have little effect on rumen pH after several days. Briggs *et al.* (1957) found that the accumulation of lactic acid was reduced if starch had been previously fed in smaller amounts, and a period of adjustment may be required for bacteria capable of converting lactic to volatile acids to become established (Elsden 1945; Johns 1949). Evidently, under the conditions of the present experiment, lactic acid was rapidly metabolised in the rumen.

Despite the large range in VFA content of silages 5–14, ruminal VFA levels were remarkably constant. Furthermore, differences in peak levels, for silages 1–4 are not related to differences in silage VFA composition. If, however it is assumed that each molecule of lactic

acid and reducing sugar is rapidly converted to one and two molecules respectively of VFA, the *potential* VFA contents of the various silages are much more uniform (Table 2, last column). The similarity of ruminal VFA levels are much more explicable from this point of view.

Silages 5–14 present an extreme range in ammonia and in RN content, but much more uniform values are obtained by combining these figures. There is no apparent reason why ruminal ammonia on silage 5 should be so high, but it is noteworthy that silages 11 and 12, with low values for ammonia plus RN, gave very low ruminal ammonia levels. Ruminal RN values were much higher and PN values lower than in grass-fed sheep, as would be expected from silage analysis.

The range in total numbers and gross morphological types of bacteria does not indicate any major influence of silage composition but it is possible that large species differences could have been induced. Average values for sheep in pens fed grass dried from the same pasture, shown at the bottom of Table 3, were higher for total count but comparable for the differential count.

The ruminal constituents measured show little correlation with silage digestibility. This may be because the fermentation curves are dependent more on the solubility in rumen fluid of the digestible constituents than on their total amount.

ACKNOWLEDGEMENTS

We particularly wish to thank Mr. R. J. Lancaster for the analyses of the silage and Mr. P. P. McKitterick for technical assistance.

REFERENCES

Briggs, P. K.; Hogan, J. P.; Reid, R. L. 1957: *Aust. J. agric. Res. 8:* 674-90.
Christian, K. R.; Williams, V. J. 1957: *N.Z. J. Sci. Tech. A38:* 1003-10.
Elliot, J. M.; Bennett, E.; Archibald, J. G. 1957: *J. Dairy Sci. 40:* 356-62.
Elsden, S. R. 1945: *J. exp. Biol. 22:* 51-62.
Johns, A. T. 1949: *Nature, London 164:* 620.

YEASTS FROM THE LEAVES OF PASTURE PLANTS

By M. E. di Menna, Soil Bureau, Department of Scientific and Industrial Research, Wellington

(*Received for publication, 22 December 1958*)

Summary

The yeast population upon the leaves of pasture plants in New Zealand has been investigated in relation to season, soil yeast flora, and incidence of facial eczema toxin in autumn pasture. Leaf yeasts were shown to be taxonomically distinct from soil yeasts and to vary with season but not to vary with the localities sampled. During most of the year numbers of yeasts varied between 30,000 and 3,500,000 per gramme of leaves, wet weight, but in the February-March period they reached peaks of up to 100,000,000 per gramme, making up about 1% of the weight of the leaf sample. At this time up to 90% of the yeast population consisted of red pigmented species. The pattern was altered when autumn rain produced fresh pasture growth, both the total numbers and the proportion of red species dropping. The bulk of this single group of organisms which may be present on normal pasture suggests that it may be necessary to think of pasture as a microcosm rather than as a limited number of higher plants.

Introduction

De Becze (1956) suggested that yeasts isolated from soil were largely derived from vegetation. A study of yeasts in a New Zealand soil (di Menna 1957) did not support this hypothesis, but it was thought that some knowledge of the yeast flora on the leaves of pasture plants would be useful as a check for future soil work. Accordingly, during a survey of some New Zealand soils under introduced pasture, samples of pasture plant leaves were collected from the sites examined and the yeasts on them studied. As the species pattern varied with season and appeared to be uninfluenced by the soil upon which the plants were growing or by the part of the country from which the plants were collected, the results are presented in chronological order.

In addition, as part of an investigation into the cause of facial eczema (a photosensitivity disease of herbivorous farm animals, apparently confined to New Zealand), an examination was made of a number of autumn pasture samples. In these samples the presence or absence of facial eczema toxin had been proved by guinea pig feeding tests (Evans *et al.* 1957) carried out by staff of Ruakura Animal Research Station, Department of Agriculture. These samples had necessarily been held at —15° to —20.5°C for periods of up to five months, whilst the feeding tests were being carried out, before they were cultured.

Materials

Twenty-four samples of freshly collected leaves were examined. Twelve were from Claudelands Showgrounds at Hamilton, four from Marton, five from Palmerston North, one from Paraparaumu, and two from Dunedin. The soil flora of the Dunedin pasture was not being

studied, but the samples were included to check the supposition that geographical location of the pasture had little influence upon the leaf yeasts.

Samples were collected between February 1957 and April 1958.

The samples taken from Dunedin, Paraparaumu, Marton, and the Hamilton area were floristically mixed, and consisted of leaves of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), together with varying proportions of invading herbs and grasses. Two of the Palmerston North samples were taken from a pure stand of perennial ryegrass, maintained by Plant Chemistry Division, Department of Scientific and Industrial Research, and two from a pure stand of white clover maintained by Grasslands Division, Department of Scientific and Industrial Research. The fifth Palmerston North sample was one of withered leaves of perennial ryegrass.

A number of Claudelands samples were taken at various stages of pasture growth after autumn rain. On one collection date (12 March 1957) 2 days after 2 in. of rain had fallen three samples were taken from an area which was being mown for experimental purposes by Ruakura Animal Research Station. One sample was of senescent growth, a second of day-old growth, and a third of week-old growth. At another time (19 March 1958) two samples of mowings were collected two days after a total of $\frac{1}{2}$ in. of rain had fallen. One was a top-level cut containing only young growth, and the second was a low-level cut which included some dead and withered material from the plant bases. On one of the collecting days at Marton it was possible to collect two samples, one of young growth and one of older material.

Stock did not have access to the Dunedin pasture, nor to the stand of white clover at Palmerston North. The other pastures were grazed by sheep.

Six frozen samples with a known level of facial eczema toxin had been collected in the Hamilton area, one from Matamata, three from Claudelands Showgrounds and two from Rukuhia. The Matamata sample was collected in March 1957, and the other five samples in February and March 1958.

METHODS

Some of the fresh samples from Claudelands and all of the frozen samples were mechanically mown. The remainder of the fresh samples were clipped by hand with sterile scissors from the areas from which soil samples were taken later. The clippings were put into polythene bags and cultured as soon as possible after collection. In most cases the samples took twenty-four hours to reach the laboratory.

Dilution of the fresh samples of leaves were made from 5 g weighed out with sterile precautions. The first dilution of 1/50 was held for *ca.* 15 min. with intermittent shaking by hand before serial dilutions were made by tenfold steps from 1/1000 to 1/10,000,000.

Straka and Stokes (1957) found that rapid and extensive destruction of bacteria in natural mixed populations in prepared foods occurred in

TABLE 1. EFFECT OF PRE-CULTURAL TREATMENTS UPON YEASTS ISOLATED FROM PASTURE PLANT LEAVES

Date Collected	Treatment	*S. roseus*	*S. pararoseus* Olson et Hammer	*B. alba*	*R. glutinis*	*R. mucilaginosa*	*R. marina*	*R. graminis*	*C. laurentii*	*C. albidus*	*C. luteolus* (Saito) Skinner	*T. ingeniosa*	*Candida albicans*	Unknown	Yeasts/g leaves
17 Dec. 57	Standard	.	.	.	.	4	8	4	76	.	.	.	.	8	100,000
	0.1% peptone water as diluent	4	.	.	8	.	8	8	48	.	.	4	12	8	90,000
	Standard	12	.	4	.	.	4	8	68	.	.	.	.	4	1,560,000
	0.1% peptone water as diluent	4	.	.	.	8	8	8	68	.	.	.	.	4	1,640,000
14 Jan. 58	Held in frozen storage 6 mths.	4	2	.	2	.	12	.	66	2	2	.	.	10	11,000,000
	Frozen storage 6 months, 1° dilution macerated	2	.	.	4	2	4	.	74	4	2	.	.	8	10,000,000

"Standard" treatment: Leaves freshly collected, tap water used as diluent, primary dilution shaken by hand. Proportions of species are expressed as percentages.

B. = *Bullera*, *C.* = *Crytococcus*, *R.* = *Rhodotorula*, *S.* = *Sporobolomyces*, *T.* = *Torulopsis*.

a number of commonly used diluents—saline, tap water, and distilled water—but that 0.1% peptone water gave protection. In order to find whether leaf yeasts were killed by the sterile tap water used as a diluent in this work, parallel dilutions from the same grass sample were made, one with tap water and one with 0.1% (w/v) peptone water, on two occasions. As no consistent differences could be found between the results from the two treatments (Table 1), it was assumed that, at least, tap water had no more harmful effect upon the suspended yeast cells than had peptone water.

A slightly different method of preparation was used for the dilutions of frozen grass. The primary dilutions of 5 g of leaves with 250 ml of sterile tap water were macerated for 2 min. in a sterilised Townsend and Mercer top drive macerator and the usual dilutions then made. To find the effect of frozen storage upon the leaf yeast population, half the pasture sample collected at Claudelands on 14 January 1958 was cultured immediately and half held at —5° to —20.5°c for six months and then cultured. Two series of dilutions were made from the frozen half of the sample, one in which the primary dilution was shaken in the manner used for fresh samples, and a second in which the primary dilution was macerated. Results from these treatments are given in Table 1. The total yeast count had increased by *ca.* seven times during storage but the proportions of the different species present had not changed, allowance being made for slight variation within the sample. The results when the primary dilution was macerated were very similar, both quantitatively and qualitatively, to those when it was shaken.

Cultures were made in the manner used for the isolation of yeasts from soil (di Menna 1957). Inocula of 0.5 ml of dilution were spread upon the surfaces of 10 cm plates of glucose peptone agar (4% glucose, 1% peptone, 2% agar) which had been brought to a pH of 4. The poured plates had been dried out for some time in an oven at 70°c before inoculation so that the inocula would be absorbed. Five plates per dilution were inoculated. The plates were incubated at room temperature for 4–6 days before reading. No bacteria grew upon the medium. Little interference was experienced from moulds, for relatively few grew from the leaf washings and many of those which appeared were of genera which produce restricted colonies. Fifty yeasts per sample were picked from the plates in a systematic manner and subcultured for identification.

Identifications were made by the criteria of Lodder and Kreger-van Rij (1952). *Pullularia pullulans* (De Bary et Low) Berkhout was treated as a yeast. Two commonly occurring species, *Rhodotorula graminis* (di Menna 1958a) and *Torulopsis ingeniosa* (di Menna 1958b), were hitherto unrecorded. Isolates of *T. ingeniosa* from soil (di Menna 1955) and from leaves (di Menna 1957) have been described previously as *Torulopsis aeria* (Saito) Lodder, which this species resembles rather closely. However, differences in cell shape and in some biochemical characters finally made it advisable to transfer oval-celled, strongly proteolytic strains to the separate species, *T. ingeniosa*.

TABLE 2. Yeasts Isolated from Freshly Collected Leaves of Pasture Plants

Date	Place	Description	*H. vinae*	*S. roseus*	*S. odorus* Derx	*B. alba*	*R. glutinis*	*R. mucilaginosa*
1957								
5 Feb.	Marton		.	.	.	.	.	.
21 Feb.	Claudelands		.	10	.	.	20	.
12 Mar.	Claudelands	Old growth	.	6	.	.	22	.
12 Mar.	Claudelands	Week old growth	.	4	.	.	8	6
12 Mar.	Claudelands	Day old growth	.	4	.	.	6	2
8 May	Marton	Old growth	.	.	.	.	.	.
8 May	Marton	Young growth	.	.	.	.	.	.
2 July	Claudelands		.	.	.	.	.	16
8 Aug.	Palmerston Nth.	Per. rye	.	.	.	.	.	.
8 Aug.	Palmerston Nth.	W. clover	.	.	.	6	.	2
3 Oct.	Marton		.	.	.	.	.	.
22 Oct.	Claudelands		.	2	.	2	.	2
2 Dec.	Palmerston Nth.	Per. rye	.	.	.	.	.	2
2 Dec.	Palmerston Nth.	W. clover	.	.	24	8	.	.
17 Dec.	Claudelands		.	2	.	.	4	2
1958								
14 Jan.	Claudelands		.	8	.	2	.	2
18 Feb.	Claudelands		.	2	.	.	4	10
26 Feb.	Dunedin		.	6	.	.	2	8
19 Mar.	Claudelands	Low-level cut	.	2	.	2	2	.
19 Mar.	Claudelands	Top-level cut	2	.	.	.	6	.
24 Mar.	Claudelands		.	2	.	.	12	.
1 Apr.	Dunedin		.	4	.	4	.	6
28 Apr.	Palmerston Nth.	Dead rye	.	2	.	.	.	6
29 Apr.	Paraparaumu		.	.	.	.	10	2

Proportions of different species present are expressed as percentages. Fifty yeasts from twenty-five yeasts from each of these two samples were identified. Where *B.* = *Bullera, Can.* = *Candida, C.* = *Cryptococcus, H.* = *Hanseniospora,*

Results

Results from the fresh samples are given in Table 2. Because there was no evidence that the yeast flora of pasture plant leaves varied with locality, they are tabulated in chronological order.

Species isolated. Twenty-one species of yeasts were isolated, but of these only eight occurred regularly, *Sporobolomyces roseus* Kluyver et van Niel, *Rhodotorula glutinis* (Fres.) Harrison, *R. mucilaginosa* (Jorg.) Harrison, *R. marina* Phaff, Mrak et Williams, *R. graminis, Bullera alba* Derx, *Cryptococcus laurentii* (Kufferath) Skinner, and *Torulopsis ingeniosa.* The remaining species were isolated from three or less of the twenty-four fresh samples. Five isolates of fermenting species, less than 0.5% of the total, were recovered; one isolate of *Hanseniospora vinae* van der Walt et Tscheuschner, three of *Candida albicans* (Robin) Berkhout,

R. flava (Saito) Lodder	*R. marina*	*R. texensis* Phaff, Mrak, et Williams	*R. macerans* Frederiksen	*R. graminis*	*C. laurentii*	*C. albidus*	*C. luteolus*	*C. diffluens*	*T. ingeniosa*	*Can. albicans*	*Can. humicola*	*Can. scottii* Diddens et Lodder	*Can. curvata*	*P. pullulans*	Unknown	Yeasts/g leaves (wet weight)
.	.	.	.	74	.	.	.	.	16	.	.	.	.	4	6	Not done
.	.	.	.	62	4	.	.	.	.	.	.	.	.	4	.	32,000,000
.	.	.	.	56	10	.	.	.	.	.	2	.	.	.	4	100,000
.	2	.	2	26	52	.	.	.	.	.	.	.	.	.	.	80,000
.	4	.	.	20	62	.	.	.	2	.	.	.	.	.	.	800,000
.	4	.	.	24	28	.	.	.	44	.	.	.	.	.	.	2,100,000
.	28	.	.	8	48	.	.	8	8	.	.	.	.	.	.	800,000
.	.	.	.	4	24	.	.	.	42	.	.	.	6	.	8	130,000
.	8	.	.	.	36	.	.	2	38	.	.	.	.	.	16	2,000,000
.	2	.	.	.	78	.	.	.	2	.	.	.	.	.	10	3,500,000
.	.	.	.	.	70	2	.	.	14	.	.	.	.	.	14	280,000
6	.	.	.	.	72	.	.	.	12	.	.	.	.	.	4	560,000
.	6	.	.	4	54	2	.	.	28	.	.	.	2	.	2	57,000
.	.	.	.	.	68	.	.	.	.	.	.	.	.	.	.	31,000
.	8	.	.	8	62	.	.	.	2	6	.	.	.	.	8	100,000
.	8	.	.	8	68	.	.	.	.	.	.	.	.	.	4	1,600,000
.	4	2	.	8	62	.	2	.	4	.	.	.	.	.	2	630,000
.	12	.	2	20	40	.	.	.	6	.	.	2	.	.	2	1,000,000
.	50	.	.	14	30	.	.	.	.	.	.	.	.	.	.	13,000,000
.	58	.	.	12	22	.	.	.	.	.	.	.	.	.	.	100,000,000
.	6	.	.	54	26	.	.	.	.	.	.	.	.	.	.	15,000,000
16	.	.	.	12	28	.	.	.	4	.	.	.	.	24	2	970,000
.	.	.	.	24	66	.	.	.	2	.	.	.	.	.	.	440,000
.	4	.	.	2	80	.	.	.	2	.	.	.	.	.	.	400,000

each sample were identified, except for the two Marton samples of 8th May, 1957. Only no description of sample is given leaves were collected at random.
P. = *Pullularia*, *R.* = *Rhodotorula*, *S.* = *Sporobolomyces*, *T.* = *Torulopsis*.

and one of an unidentified species. The significance of the occurrence of the animal pathogen, *C. albicans*, has been discussed already (di Menna 1958c).

Forty-one isolates did not correspond with any published descriptions. None of the unidentified strains were ascosporogenous or pigmented; they appeared to belong to a number of *Torulopsis* and *Candida* spp.

The proportions of red-pigmented *Sporobolomyces* and *Rhodotorula* spp. varied seasonally (Fig. 1). Red-pigmented species made up *ca.* 80% of the isolates from leaves collected from Marton and Claudelands in February 1957 and from the sample of senescent growth collected from Claudelands on 12 March 1957. Proportions of red species were low on the two samples of young growth collected from Claudelands on

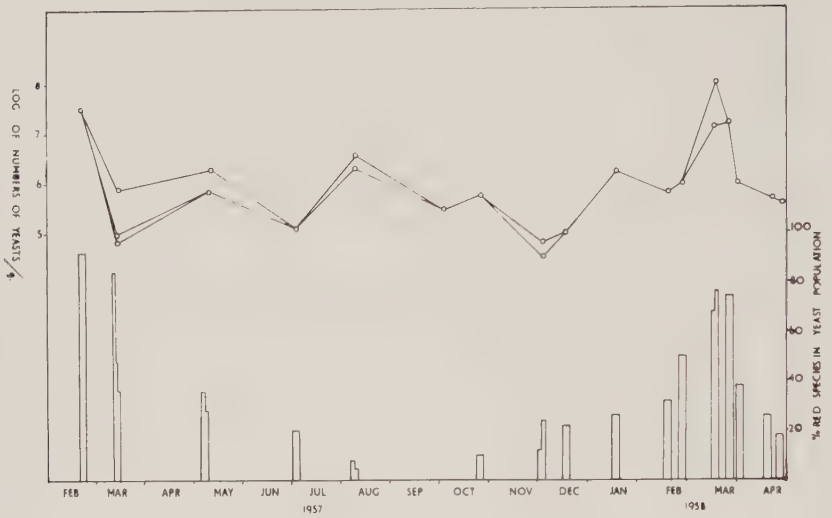


Fig. 1.—Quantitative and qualitative seasonal changes in yeasts on leaves of pasture plants. When more than one sample was taken on a single day the line graph is split and the histogram stepped.

12 March 1957 and on samples collected during the next five months. By August very few red yeasts were recoverable. At the beginning of the summer of 1957-58 percentages increased again, and a peak was reached in March 1958 when red yeasts made up over 70% of the isolates from samples collected from Claudelands . In April 1958, proportions dropped again.

Of the five regularly occurring red species only *R. marina* and *R. graminis* were dominant in any of the samples. *R. graminis* was dominant in the February 1957 samples from Marton and from Claudelands, in one sample from Claudelands taken in March 1957, and in a Claudelands sample collected 24 March 1958. *R. marina* was dominant in both the top- and the low-level cut taken from Claudelands on 19 March 1958.

The proportions of *C. laurentii* were the inverse of those of the red yeasts, low in late summer to early autumn, high during the winter and spring. The occurrence of *T. ingeniosa* was sporadic. It made up about 40% of the isolates from a number of winter samples but did not appear regularly in high percentages. It was often absent from summer and autumn samples.

Numbers of yeasts. Numbers of yeasts per gramme of leaves, wet weight, varied between 100,000,000 on the top-level cut from Claudelands, taken 19 March 1958, and 31,000 on white clover collected at Palmerston North on 2 December 1957. The sample of perennial ryegrass collected from Palmerston North, also on 2 December 1957, had the second-lowest count of 57,000 yeasts per gramme. A peak in numbers associated with a dominance of red species in late summer or

early autumn seems to be characteristic, for the second highest count, of 32,000,000 yeasts per gramme of leaves, was recorded from the Claudelands sample of 21 February 1957. Although the counts made between these two peaks, in February 1957 and in March 1958, were all less than 3,500,000/g, there was not a regular fall and rise between peak and peak but an erratic variation.

Relation between Leaf and Soil Yeasts

The yeasts associated with the leaves of introduced pasture plants in New Zealand are taxonomically distinct from those common in soil (di Menna 1957, 1958d), although physiologically very similar (di Menna 1959). The pattern of the two floras is influenced by different factors: the leaf flora is affected by season, both quantitatively and qualitatively, and not apparently by locality; the soil flora varies much with locality but little with season.

Of the yeasts isolated from leaves, only *Cryptococcus albidus* (Saito) Skinner, *Cryptococcus diffluens* (Zach) Lodder et Kreger-van Rij, *Candida humicola* (Daszewska) Diddens et Lodder, and *Candida curvata* (Diddens et Lodder) Lodder et Kreger-van Rij, have so far been found commonly and in large proportions in New Zealand soils. These species were present in leaf samples on only one or two occasions and it is likely that they were the result of contamination from splashings of soil or worm casts. Conversely, the numbers of commonly occurring leaf yeasts found in soil at a depth of 1 in. or more have been small, although these species must reach the soil in large numbers, washed from the leaves by rain or on plant debris. Although the upper soil layers may act as a filter to rainwater suspensions of leaf yeasts, this cannot be enough to prevent their eventual distribution throughout the lower layers by movements of the soil fauna or on fungus mycelium. It is therefore necessary to postulate that biological factors prevent leaf yeasts from establishing themselves in soil.

Factors Affecting Leaf Yeasts

When pasture-plant leaves are examined microscopically, small colonies of yeasts can usually be seen about the borders of some of the epidermal cells (Fig. 2), where apparently they multiply in nutrient exudates which collect in these depressions. Factors affecting these yeasts would be physical and chemical, due to weather, climate, and plant.

The effectiveness of the washing technique in removing yeasts from leaves suggests that rain will cause short-term reductions in population. Long-term changes are influenced by season directly or by the effects of season upon the plant. The summer increase in proportions of red species continues until February or March and their dominance is probably related to rise in temperature rather than to increase in light intensity.

Detailed knowledge of changes in plant exudates with age of plant is lacking, but it seems reasonable to suppose that nutrients available on withered or senescent leaves would differ from those on young leaves, and that kind and amount of nutrients would influence kind and

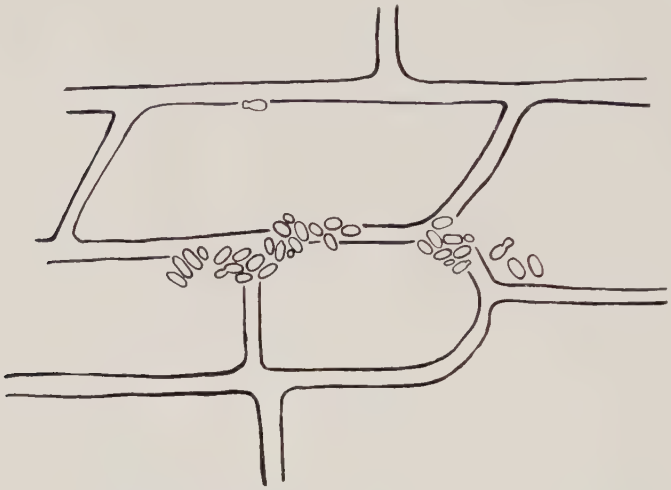


Fig. 2.—Yeasts clustered about edges of epidermal cells of white clover leaves. ×500.

numbers of species present. The March 1957 collections from Claudelands suggested that this was so. Old growth collected after rain supported a flora qualitatively similar to that on old growth before rain, although much reduced in numbers. Young growth collected at the same time after rain supported a flora which was qualitatively different.

On the other hand there is evidence which appears to contradict this. There was very little difference between the kinds of yeasts isolated from the top-level cut from Claudelands taken 19 March 1958, which contained only young growth, and those from the low-level cut taken from the same place on the same day which included withered material. Similarly, the yeast flora on withered leaves collected from Palmerston North on 28 April 1958 resembled that on vigorous growth collected from Paraparaumu on the following day.

Detailed meteorological data were available for the Hamilton and Palmerston North areas, less detailed for Marton, Paraparaumu, and Dunedin. No correlation could be obtained between the yeast numbers on the leaves and evaporimeter and anemometer readings, humidities, temperatures, rainfall, and hours of sun during the seven-day periods before sampling, when these factors were considered singly. It is probable that a combination of these factors, together with the modification that density and height of the pasture plants would have upon humidity and light intensity, is significant, but, to establish this and the effect of the age of the leaves, work is needed on ungrazed plots of single pasture species situated within a short distance of a microclimate station.

Herbivore : Leaf-Yeast Relationship

As much of this work was done as part of an investigation into the cause of facial eczema, it is of interest to record the relationships examined.

TABLE 3. YEASTS ISOLATED FROM FROZEN SAMPLES OF PASTURE LEAVES
(Percentage of species)

Date Collected	Locality collected	Toxicity	*S. roseus*	*B. alba*	*R. glutinis*	*R. mucilaginosa*	*R. marina*	*R. macerans*	*R. graminis*	*C. laurentii*	*C. albidus*	*T. ingeniosa*	*Can. parapsilosis* (Ashf.) Langeron et Tali.	Unknown	Yeasts/g leaves
1958 22nd Feb.	Rukuhia	Non-toxic	.	2	2	2	16	.	.	70	4	2	.	2	14,000,000
13th Mar.	Claudelands	Non-toxic	2	.	20	2	6	2	50	14	2	.	.	2	330,000,000
15th Mar.	Claudelands	Mildly toxic	.	.	4	2	38	.	34	22	.	.	.	.	1,000,000
13th Mar.	Claudelands	Severely toxic	.	.	2	4	30	.	12	50	.	.	2	.	23,000,000
14th Mar.	Rukuhia	Severely toxic	4	.	.	.	4	.	14	78	.	.	.	.	600,000
1957 16th Mar.	Matamata	Toxic	4	.	.	.	6	.	18	64	.	6	.	2	1,800,000

Level of facial eczema toxin determined by guinea pig feeding tests.

The results from the frozen grass samples of known degree of toxicity (Table 3) suggested that the period of production of facial eczema toxin coincides with a time of change in the leaf yeast population, when the large proportions of red yeasts, which have reached a peak by steady increments during the summer, begin to drop and when total numbers also drop.

In the three Claudelands samples of known degree of toxicity collected within a 48-hour period (13-15 March 1958), the ratio of red : colourless yeasts was greater in the non-toxic than in the severely toxic sample and was intermediate in the sample of low toxicity. Proportions of red yeasts in the severely toxic sample from Rukuhia collected 14 March 1958, and in the Matamata toxic sample of 16 March 1957, were low. The non-toxic sample from Rukuhia contained only 18% of red yeasts, but had been collected a month earlier than the other frozen samples, on 22 February 1958, and was not comparable with them but rather with the fresh sample collected from Claudelands on 18 February 1958, which contained 30% of red species.

Although yeasts proved to be unconnected with facial eczema, it is of more than passing interest that the yeast content of pasture leaves eaten by herbivores may vary from almost nothing in the spring to about 1% by weight in the early autumn (1 g of yeast culture harvested from a solid medium contains *ca.* 7×10^9 cells; leaves in the autumn support yeast populations of up to 10^8 cells per gramme). Micro-organisms occurring in such proportions must to some extent alter the total chemical composition of herbage, either by catabolism, destroying or modifying plant products, or by anabolism, synthesising substances such as vitamins, pigments, antibiotics, microbial fats, proteins, and carbohydrates, which will be included in the animal diet. These alterations will be seasonal, dependent upon the ebb and flow of the film of micro-organisms on the leaf surfaces. It seems it is not safe to regard grass as grass alone; it must be thought of as a microcosm.

Acknowledgements

I am indebted to Mr. J. C. Percival, Ruakura Animal Research Station, Department of Agriculture; to Dr. K. J. Mitchell, Grasslands Division, Department of Scientific and Industrial Research; to Dr. G. W. Butler, Plant Chemistry Division, Department of Scientific and Industrial Research; and to Professor G. T. S. Baylis, and Mrs. J. B. Hamel, of the Department of Botany, University of Otago; for providing me with much of the material for this work.

References

Becze, G. I. de 1956: Yeasts. I. Morphology. *Appl. Microbiol. 4:* 1.

Evans, J. V.; McFarlane, D.; Reid, C. S. W.; Perrin, D. E. 1957: Photosensitivity Diseases in New Zealand. IX. The Susceptibility of the Guinea Pig to Facial Eczema. *N.Z. J. Sci. Tech. A38:* 491.

Lodder, J.; Kreger-van Rij, N. J. W. 1952: "The Yeasts: A Taxonomic Survey." North-Holland Publishing Co., Amsterdam.

Menna, M. E. di 1955: A Search for Pathogenic Species of Yeasts in New Zealand Soils. *J. gen. Microbiol. 12:* 54.

———1957: The Isolation of Yeasts from Soil. *Ibid. 17:* 678.
———1958a: Two New Species of Yeasts from New Zealand. *Ibid. 18:* 269.
———1958b: *Torulopsis ingeniosa* n. sp. from Grass Leaves. *Ibid. 19:* 581.
———1958c: *Candida albicans* from Grass Leaves. *Nature, Lond., 181:* 1287.
———1958d: Biological Studies of Some Tussock Grassland Soils. III. Yeasts. *N.Z. J. agric. Res. 1:* 939.
———1959: Some Physiological Characters of Yeasts from Soils and Allied Habitats. *J. gen. Microbiol. 20:* 13.
Straka, R. P.; Stokes, J. L. 1957: Rapid Destruction of Bacteria in Commonly Used Diluents and its Elimination. *Appl. Microbiol. 5:* 21.

FOURTH SUPPLEMENT TO "A REVISED LIST OF PLANT DISEASES RECORDED IN NEW ZEALAND", 1957-1958

By R. M. Brien and Joan M. Dingley, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(*Received for publication, 5 December 1958*)

Introduction

In this Fourth Supplement (to *N.Z. Dep. sci. industr. Res. Bull. 101*) are listed 95 diseases on 114 host plants. It includes new records, additional host records, and diseases recorded in publications since the preparation of the Third Supplement (Brien and Dingley 1957). New records include *Alternaria dianthicola* on *Dianthus caryophyllus* and *Fusarium bulbigenum* var. *batatas* on *Ipomoea batatas*, while additional host records contain *Phytophthora cinnamomi* on a number of host plants and *Puccinia iridis* on nine species of *Iris*. Diseases of some importance which have been recorded since the Third Supplement are *Ustilago violaceae*, *Septoria passiflorae*, shell-bark virus, citrus exocortis virus and citrus tristeza virus.

We are indebted to Dr. F. J. Newhook of this Division for identification of the *Phytophthora* spp. listed in this Supplement.

Agathis australis Salisb.	
Pythium ultimum Trow.	Additional host record
Phytophthora cinnamomi Rands	Additional host record
Phytophthora parasitica Dastur	Additional host record
Acacia verticillata Willd.	
Phytophthora cinnamomi Rands	Additional host record
Aesculus hippocastanum L.	
Stereum purpureum (Pers.) Fr.	Additional host record
Agropyron repens (L.) Beauv.	
Urocystis agropyri (Preuss) Schroet.	Brook, S. D. 1957
Agropyron scabrum (Labill.) Beauv.	
Ustilago hypodytes (Schlecht.) Fr.	Brook, S. D. 1957
Albizzia lophantha Benth.	
Armillaria mellea (Fr.) Quel.	Additional host record
Allium fistulosum L.	
Pleospora herbarum (Pers. ex Fr.) Rabenh.	Additional host record
Peronospora destructor (Berk.) Casp.	Additional host record
Alopecurus geniculatus L.	
Scolecotrichum graminis Fckl.	Additional host record
Anthoxanthum aristatum Boiss.	
Tilletia anthoxanthi Blytt	Brook, S. D. 1957
Arrhenatherum elatius (L.) Mert. et Koch.	
Scolecotrichum graminis Fckl.	Additional host record
Banksia sp.	
Phytophthora cinnamomi Rands	Additional host record
Beta vulgaris L.	
Fusarium semitectum Berk. et Rav.	New record
Boronia megastigma Nees	
Phytophthora cinnamomi Rands	Additional host record
Brassica napo-brassica DC.	
Fusarium conglutinans Wollenw.	New record
Brassica oleracea var. **gemmifera** Zenk.	
Sclerotinia sclerotiorum (Lib.) de Bary	Additional host record

Brassica oleracea var. **ramosa** Alef.	
Peronospora parasitica (Pers.) Tul.	Additional host record
Brassica rapa L.	
Fusarium conglutinans Wollenw.	New record
Bromus catharticus Vahl	
Colletotrichum graminicola (Ces.) Wilson	Dingley 1959
Camellia sasanqua Thunb.	
Exobasidium camelliae Shirai	Additional host record
Pestalotia guepini Desm.	Additional host record
Carex pseudocyperus L.	
Farysia olivacea (DC.) H. et P. Sydow	Brook, S. D. 1957
Citrus aurantifolia Swingle	
Citrus tristeza virus—stem pitting strain of	Fletcher 1957a
Citrus limon (L.) Burm. f.	
Shell-bark virus	Fletcher 1957a
Citrus paradisi Macf.	
Citrus tristeza virus—stem pitting strain of	Fletcher 1957a
Citrus sinensis Osbeck	
Phytophthora citrophthora (Smith et Smith) Leon.	Fletcher 1957b
Citrus sp. (citronelle)	
Alternaria citri Pierce	Dingley 1959
Phytophthora citrophthora (Smith et Smith) Leon.	Fletcher 1957b
Cladium glomeratum R. Br.	
Phyllachora cladii-glomerati Hansf.	Hansford 1957
Coprosma repens A. Rich.	
Puccinia coprosmae Cke.	Additional host record
Coronopus didymus (L.) Sm.	
Albugo candida Hook.	Additional host record
Crepis capillaris L.	
Ramularia eximia Bubak	Dingley 1959
Cucurbita pepo L.	
Sphaerotheca fuliginea (Schlecht.) Poll.	Dingley 1959
Cynodon dactylon (L.) Pers.	
Ustilago cynodontis (Pass.) P. Henn.	Brook, S. D. 1957
Dactylis glomerata L.	
Dilophospora alopecuri Fr.	Additional host record
Rhynchosporium orthosporum Caldwell	Latch and Wenham 1958
Scolecotrichum graminis Fckl.	Latch and Wenham 1958
Selenophoma donacis (Pass.) Sprague et Johnson	Latch and Wenham 1958
Stagonospora maculata (Grove) Sprague	Latch and Wenham 1958
Dianthus caryophyllus L.	
Alternaria dianthicola Neergard	New record
Ustilago violaceae (Pers.) Roussl.	Robinson 1957a
Verticillium dahliae Kleb.	Robinson 1957b
Dodonaea viscosa Jacq.	
Pythium ultimum Trow.	Additional host record
Verticillium dahliae Kleb.	Additional host record
Eriobotrya japonica Lindl.	
Phyllosticta eriobotryae Thuem	New record
Physalospora obtusa (Schw.) Cke.	Additional host record
Euonymus europaeus L.	
Armillaria mellea (Fr.) Quel.	Additional host record
Festuca elatior L.	
Urocystis agropyri (Preuss) Schroet.	Brook, S. D. 1957
Ficus carica L.	
Ascochyta caricae Rabenh.	New Record

Gerbera jamesoni Bolus	
Fusarium oxysporum Schlecht. ex Fr.	Robinson 1957b
Phytophthora cryptogea Pethybr. et Laff.	Robinson 1957b
Glyceria fluitans (L.) R. Br.	
Ustilago longissima (Schlecht.) Meyer	Brook, S. D. 1957
Hermodactylus tuberosus Salisb.	
Puccinia iridis Wallr.	Salinger 1958
Holcus mollis L.	
Colletotrichum graminicola (Ces.) Wilson	Dingley 1959
Tilletia holci (Westend.) Schroet.	Brook, S. D. 1957
Humulus lupulus L.	
Molybdenum deficiency	Askew *et al.*, 1958
Ipomoea batatas (L.) Lam.	
Fusarium bulbigenum var. *batatas* Wr.	New record
Rhizopus stolonifer (Ehrenb. ex Fr.) Lind	Additional host record
Iris chrysographes Dykes	
Puccinia iridis Wallr.	Additional host record
Iris fulva Ker	
Puccinia iridis Wallr.	Salinger 1958
Iris fulvala Dykes	
Puccinia iridis Wallr.	Salinger 1958
Iris germanica L.	
Puccinia iridis Wallr.	Additional host record
Sclerotium rolfsii Sacc.	Salinger 1958
Erwinia carotovora (Jones) Holland	Salinger 1958
Iris hexagona Walt. (Louisiana hybrids)	
Puccinia iridis Wallr.	Salinger 1958
Iris laevigata Fisch.	
Puccinia iridis Wallr.	Additional host record
Iris nepalensis D. Don	
Puccinia iridis Wallr.	Additional host record
Iris setosa Pall.	
Puccinia iridis Wallr.	Additional host record
Iris versicolor L.	
Puccinia iridis Wallr.	Additional host record
Isopogon sp.	
Phytophthora cinnamomi Rands	Additional host record
Juncus planifolius R. Br.	
Sorosporium piluliformis (Berk.) McAlp.	Brook, S. D. 1957
Kentranthus ruber (L.) DC.	
Ramularia centranthi Brun.	Dingley 1959
Lactuca sativa L.	
Olpidium brassicae (Woron.) Dang.	Fry 1958
Pellicularia filamentosa (Pat.) Roger	Brien *et al.* 1957
Pythium ultimum Trow.	Brien *et al.* 1957
Lathyrus latifolius L.	
Ramularia deusta (Fckl.) Baker, Snyder et Davis	Dingley 1959
Lathyrus pratensis L.	
Ramularia deusta (Fckl.) Baker, Snyder et Davis	Dingley 1959
Leptospermum scoparium Forst.	
Phomatospora leptospermi Hansf.	Hansford 1957
Leucodendron adscandens R. Br.	
Phytophthora cinnamomi Rands	Additional host record
Leucopogon fasciculatus A. Rich.	
Vestergrenia leucopogonis Hansf.	Hansford 1957
Lolium perenne L.	
Colletotrichum graminicola (Ces.) Wilson	Dingley 1959

Luculia gratissima Sweet
Phytophthora sp. — Anon. 1957a
Lupinus angustifolius L.
Pleiochaeta setosa (Kirchner) Hughes — Dingley 1959
Phytophthora cinnamomi Rands — Additional host record

Malus sylvestris (L.) Mill.
Botryosphaeria ribis Gross. et Dugg. — Additional host record
Gloeosporium album Osterw. — Brook, P. J. 1957
Verticillium dahliae Kleb. — Additional host record
Iron deficiency — Anon. 1957b
Medicago sativa L.
Pleospora herbarum (Pers. ex Fr.) Rabenh. — Additional host record
Metrosideros umbellata Cav.
Meliolina novae-zelandiae Hansf. — Additional host record

Nicotiana tabacum L.
Botrytis cinerea Pers. — Anon. 1957c
Pythium de baryanum Hesse — Additional host record
Pythium ultimum Trow. — Additional host record
Nothofagus menziesii (Hook. f.) Oerst.
Cyttaria nigra Rawlings — Rawlings 1956
Cyttaria pallida Rawlings — Rawlings 1956

Papaver somniferum L.
Entyloma fuscum Schroet. — Brook, S. D. 1957
Parietaria debilis Forst. f.
Entyloma parietariae Rayss — Brook, S. D. 1957
Paspalum dilatatum Poir.
Colletotrichum graminicola (Ces.) Wilson — Dingley 1959
Passiflora edulis Sims
Septoria passiflorae Louw — Dingley 1959
Petroselinum crispum (Mill.) Nym.
Septoria petroselini Desm. — Dingley 1959
Phalaris minor L.
Puccinia coronata Cda. — Additional host record
Phleum pratense L.
Scolecotrichum graminis Fckl. — Latch and Wenham 1958
Phoenix canariensis Chabaud
Graphiola phoenicis (Moug.) Poit. — Dingley 1959
Picris echioides L.
Ramularia picridis Fautr. et Roum. — Dingley 1959
Pinus radiata D. Don
Lophodermium pinastri (Schrad. ex Fr.) Chev. — Rawlings 1957
Phytophthora cactorum (Leb. et Cohn) Schroet. — Newhook 1958
Phytophthora megasperma Drechsler — Newhook 1958
Stereum sanguinolentum (Alb. et Schw.) Fr. — Cunningham 1956
Pittosporum crassifolium A. Cunn.
Armillaria mellea (Fr. Quel.) — Additional host record
Plantago lanceolata L.
Ramularia rhabdospora (Berk. et Br.) Nannf. — Dingley 1959
Poa caespitosa Forst.
Ustilago hypodytes (Schlecht.) Fr. — Brook, S. D. 1957
Ustilago striiformis (Westend.) Niessl — Brook, S. D. 1957
Polygonum hydropiper L.
Ustilago anomala J. Kunze — Brook, S. D. 1957
Polygonum sp.
Ustilago anomala J. Kunze — Brook, S. D. 1957
Poncirus trifoliata Raf.
Phytophthora citrophthora (Smith et Smith) Leon. — Fletcher 1957b
Citrus exocortis virus — Fletcher 1957a
Citrus tristeza virus—stem pitting strain of — Fletcher 1957a

Poncirus trifoliata × **Citrus sinensis** Osb. (Morton's citrange)	
Citrus tristeza virus—stem pitting strain of	Fletcher 1957a
Populus tremula L.	
Stereum purpureum (Pers.) Fr.	Cunningham 1956
Prostanthera sp.	
Phytophthora sp.	Anon. 1957a
Prunus persica (L.) Batsch	
Peach calico	Chamberlain, *et al.* 1957
Pseudotsuga taxifolia (Poir.) Britt.	
Stereum sanguinolentum (Alb. et Schw.) Fr.	Cunningham 1956
Rhododendron sp. cult.	
Cercospora handelii Bubak	McArthur 1959
Glomerella cingulata (Stonemn.) Spauld. et V. Schr.	Additional host record
Stereum purpureum (Pers.) Fr.	Additional host record
Rosa sp. cult.	
Botryosphaeria ribis Gross. et Dugg	Additional host record
Rubus idaeus L.	
Phytophthora cactorum (Leb. et Cohn) Schroet.	Anon. 1958
Phytophthora cinnamomi Rands	Anon. 1958
Rubus sp. (boysenberry)	
Pseudomonas syringae van Hall	Anon. 1957a
Rumex crispus L.	
Ramularia rubella (Bon.) Nannf.	Dingley 1959
Rumex obtusifolius L.	
Ramularia rubella (Bon.) Nannf.	Dingley 1959
Sagina apetala L.	
Ustilago duriaeana Tul.	Brook, S. D. 1957
Schoenus maschalinus R. et S.	
Cintractia schoenus G. H. Cunn.	Brook, S. D. 1957
Senecio cruentus DC.	
Albugo tragopogonis S. F. Gray	Additional host record
Sisymbrium altissimum L.	
Albugo candida Hook.	Additional host record
Solanum laciniatum Ait.	
Phytophthora infestans (Mont.) de Bary	Driver 1957
Solanum simile Muell.	
Phytophthora infestans (Mont.) de Bary	Driver 1957
Sorbus aucuparia L.	
Gloeosporium sorbi Rostr.	New record
Sopergula arvensis L.	
Olpidium brassicae (Woron.) Dang.	Fry 1958
Stellaria media (L.) Vill.	
Olpidium brassicae (Woron.) Dang.	Fry 1958
Stellaria parviflora Banks et Soland.	
Puccinia arenariae (Schum.) Wint.	Jorstad 1957
Syringa vulgaris L.	
Glomerella cingulata (Stonemn.) Spauld et V. Schr.	Additional host record
Taraxacum officinale Weber	
Ramularia taraxaci Karst.	Dingley 1959
Tecomanthe speciosa Oliver	
Glomerella cingulata (Stonemn.) Spauld. et V. Schr.	Additional host record
Trifolium repens L.	
Colletotrichum destructivum O'Gara	Blair 1958
Thryptomene sp.	
Phytophthora cinnamomi Rands	Additional host record
Tricuspidaria sp.	
Verticillium dahliae Kleb.	Additional host record

Uncinia compacta R. Br.
Cintractia sclerotiformis (Cke. et Mass.) G. H. Cunn. .... Brook, S. D. 1957

Veronica tournefortii Gmel.
Olpidium brassicae (Woron.) Dang. Fry 1958
Vicia sp.
Peronospora viciae (Berk.) de Bary . Additional host record

List of Diseases

Albuga candida Hook.
Coronopus didymus
Sisymbrium altissimum
Albugo tragopogonis S. F. Gray
Senecio cruentus
Alternaria citri Pierce
Citrus sp. (citronelle)
Alternaria dianthicola Neergard
Dianthus caryophyllus
Armillaria mellea (Fr.) Quel.
Albizzia lophantha
Euonymus europaeus
Pittosporum crassifolium
Ascochyta caricae Rabenh.
Ficus carica

Botryosphaeria ribis Gross. et Dugg.
Malus sylvestris
Rosa sp. cult.
Botrytis cinerea Pers.
Nicotiana tabacum

Cercospora handelii Bubak
Rhododendron sp. cult.
Cintractia schoenus G. H. Cunn.
Schoenus maschalinus
Cintractia sclerotiformis (Cke. et Mass.) G. H. Cunn.
Uncinia compacta
Citrus exocortis virus
Poncirus trifoliata
Citrus tristeza virus—stem-pitting strain of
Citrus aurantifolia
Citrus paradisi
Poncirus trifoliata
Poncirus trifoliata × *Citrus sinensis* (Morton's citrange)
Colletotrichum destructivum O'Gara
Trifolium repens
Colletotrichum graminicola (Ces.) Wilson
Bromus catharticus
Holcus mollis
Lolium perenne
Paspalum dilatatum
Cyttaria nigra Rawlings
Nothofagus menziesii
Cyttaria pallida Rawlings
Nothofagus menziesii

Dilophospora alopecuri Fr.
Dactylis glomerata

Entyloma parietariae Rayss
Parietaria debilis
Entyloma fuscum Schroet.
Papaver somniferum
Erwinia carotovora (Jones) Holland
Iris germanica
Exobasidium camelliae Shirai
Camellia sasanqua

Farysia olivacea (DC.) H. et P. Sydow
Carex pseudocyperus
Fusarium bulbigenum var. **batatas** Wr.
Ipomoea batatas
Fusarium conglutinans Wollenw.
Brassica napo-brassica
Brassica rapa
Fusarium oxysporum Schlecht. ex Fr.
Gerbera jamesoni
Fusarium semitectum Berk. et Rav.
Beta vulgaris

Gloeosporium album Osterw.
Malus sylvestris
Gloeosporium sorbi Rostr.
Sorbus aucuparia
Glomerella cingulata (Stonemn.) Spauld. et V. Schr.
Rhododendron sp. cult.
Syringa vulgaris
Tecomanthe speciosa
Graphiola phoenicis (Moug.) Poit.
Phoenix canariensis

Iron deficiency
Malus sylvestris

Lophodermium pinastri (Schrad. ex Fr.) Chev.
Pinus radiata

Meliolina novae-zelandiae Hansf.
Metrosideros umbellata
Molybdenum deficiency
Humulus lupulus

Alpidium brassicae (Woron.) Dang.
Lactuca sativa
Spergula arvensis
Stellaria media
Veronica tournefortii

Peach calico
Prunus persica
Pellicularia filamentosa (Pat.) Rogers
Lactuca sativa
Peronospora destructor (Berk.) Casp.
Allium fistulosum
Peronospora parasitica (Pers.) Tul.
Brassica oleracea var. *ramosa*
Peronospora viciae (Berk.) de Bary
Vicia sp.
Pestalotia guepini Desm.
Camellia sasanqua
Phomatospora leptospermi Hansf.
Leptospermum scoparium
Phyllachora cladii-glomerati Hansf.
Cladium glomeratum
Phyllosticta eriobotryae Thuem.
Eriobotrya japonica
Physalospora obtusa (Schw.) Cke.
Eriobotrya japonica
Phytophthora cactorum (Leb. et Cohn) Schroet.
Pinus radiata
Rubus idaeus
Phytophthora cinnamomi Rands
Acacia verticillata
Agathis australis
Banksia sp.
Boronia megastigma
Isopogon sp.
Leucodendron adscandens
Lupinus angustifolius
Rubus idaeus
Thryptomene sp.
Phytophthora citrophthora (Smith et Smith) Leon.
Citrus sinensis
Citrus sp. (citronelle)
Poncirus trifoliata
Phytophthora cryptogea Pethybr. et Laff.
Gerbera jamesoni
Phytophthora infestans (Mont.) de Bary
Solanum laciniatum
Solanum simile
Phytophthora megasperma Drechsler
Pinus radiata
Phytophthora parasitica Dastur
Agathis australis
Phytophthora sp.
Luculia gratissima
Prostanthera sp.
Pleiochaeta setosa (Kirchner) Hughes
Lupinus angustifolius
Pleospora herbarum (Pers. ex Fr.) Rabenh.
Allium fistulosum
Medicago sativa
Pseudomonas syringae van Hall
Rubus sp. (boysenberry)
Puccinia arenariae (Schum.) Wint.
Stellaria parviflora
Puccinia coprosmae Cke.
Coprosma repens
Puccinia coronata Cda.
Phalaris minor
Puccinia iridis Wallr.
Hermodactylus tuberosus
Iris chrysographes
Iris fulva
Iris fulvala
Iris germanica
Iris hexagona
Iris laevigata
Iris nepalensis
Iris setosa
Iris versicolor
Pythium de baryanum Hesse
Nicotiana tabacum
Pythium ultimum Trow.
Agathis australis
Dodonaea viscosa
Lactuca sativa
Nicotiana tabacum

Ramularia centranthi Brun.
Kentranthus ruber
Ramularia deusta (Fckl.) Baker, Snyder, et Davis
Lathyrus latifolius
Lathyrus pratensis
Ramularia eximia Bubak
Crepis capillaris
Ramularia picridis Fautr. et Roum.
Picris echioides
Ramularia rhabdospora (Berk. et Br.) Nannf.
Plantago lanceolata L.
Ramularia rubella (Bon.) Nannf.
Rumex obtusifolius
Rumex crispus
Ramularia taraxaci Karst.
Taraxacum officinale
Rhizopus stolonifer (Ehrenb. ex Fr.) Lind
Ipomoea batatas
Rhynchosporium orthosporum Caldwell
Dactylis glomerata

Sclerotinia sclerotiorum (Lib.) de Bary
Brassica oleracea var. *gemmifera*
Sclerotium rolfsii Sacc.
Iris germanica
Scolecotrichum graminis Fckl.
Alopecurus geniculatus
Arrhenatherum elatius
Dactylis glomerata
Phleum pratense

Selenophoma donacis (Pass.) Sprague et Johnson
Dactylis glomerata
Septoria passiflorae Louw
Passiflora edulis
Septoria petroselini Desm.
Petroselinum crispum
Shell-bark virus
Citrus limon
Sorosporium piluliformis (Berk.) McAlp.
Juncus planifolius
Sphaerotheca fuliginea (Schlecht.) Poll.
Cucurbita pepo
Stagonospora maculata (Grove) Sprague
Dactylis glomerata
Stereum purpureum (Pers.) Fr.
Aesculus hippocastanum
Populus tremula
Rhododendron sp. cult.
Stereum sanguinolentum (Alb. et Schw.) Fr.
Pinus radiata
Pseudotsuga taxifolia

Tilletia anthoxanthi Blytt
Anthoxanthum aristatum
Tilletia holci (Westend.) Schroet.
Holcus mollis

Urocystis agropyri (Preuss) Schroet.
Agropyron repens
Festuca elatior
Ustilago amomala J. Kunze
Polygonum hydropiper
Polygonum sp.
Ustilago cynodontis (Pass.) P. Henn.
Cynodon dactylon
Ustilago duriaeana Tul.
Sagina apetala
Ustilago hypodytes (Schlecht.) Fr.
Agropyron scabrum
Poa caespitosa
Ustilago longissima (Schlecht.) Meyer
Glyceria fluitans
Ustilago striiformis (Westend.) Niessl
Poa caespitosa
Ustilago violaceae (Pers.) Roussl.
Dianthus caryophyllus

Verticillium dahliae Kleb.
Dianthus caryophyllus
Dodonaea viscosa
Malus sylvestris
Tricuspidaria sp.
Vestergrenia leucopogonis Hansf.
Leucopogon fasciculatus

REFERENCES

Anon. 1957a: *Annu. Rep. N.Z. Dep. Agric. 1957,* pp. 49-60.
———1957b: *Orchard. N.Z. 30(11):* 13-15.
———1957c: *Annu. Rep. Cawthron Inst. 1956–57,* pp. 16-18.
———1958: *Annu. Rep. N.Z. Dep. Agric. 1957–58,* p. 98.
Askew, H. O.; Monk, R. J.; Watson, Joyce 1958: *N.Z. J. agric. Res. 1:* 553-68.
Blair, I. D. 1958: *Canterbury Agric. Coll. Annu. Rev.* pp. 83-6.
Brien, R. M.; Dingley, J. M. 1957: *N.Z. J. Sci. Tech. A38:* 777-81.
Brien, R. M.; Dye, D. W.; Fry, P. R.; Harrison, R. A.; Jacks, H.; Newhook, F. J. 1957: *N.Z. Dep. sci. industr. Res. Inf. Ser. No. 14,* 38 pp.
Brook, P. J. 1957: *N.Z. J. Sci. Tech. A38:* 735-41.
Brook, S. D. 1957: *Trans. roy. Soc. N.Z. 84:* 643-8.
Chamberlain, E. E.; Atkinson, J. D.; Hunter, J. A. 1957: *N.Z. J. Sci. Tech. A38:* 813-9.
Cunningham, G. H. 1956: *Trans. roy. Soc. N.Z. 84:* 201-31.
Dingley, Joan M. 1959: *N.Z. J. agric. Res. 2:* 380-6.
Driver, C. M. 1957: *Nature Lond., 180 (4598):* 1367-8.
Fletcher, W. A. 1957a: *Orchard. N.Z. 30(10):* 33-6.
———1957b: *Ibid. 30(11):* 9.
Fry, P. R. 1958: *N.Z. J. agric. Res. 1:* 301-4.
Hansford, C. G. 1957: *J. Linn. Soc. N.S.W. 82:* 209-29.
Jorstad, Ivan 1957: *Nytt Mag. Bot. 5:* 23-32.
Latch, G. C. M.; Wenham, H. T. 1958: *N.Z. J. agric. Res. 1:* 182-8.
McArthur, G. W. F. Maris 1959: *N.Z. J. agric. Res. 2:* 86-9.
Newhook, F. J. 1958: *Commonw. Phytopath. News 4:* 9-10.
Rawlings, G. B. 1956: *Trans. roy. Soc. N.Z. 84:* 19-28.
———1957: *N.Z. For. Res. Inst. Tech. Pap. 20,* 16 pp.
Robinson, J. 1957a: *N.Z. Gdnr. 13:* 800-1.
———1957b: "Outdoor Carnations and Gerberas in N.Z." 72 pp. A.H. & A.W. Reed, Wellington.
Salinger, J. P. 1958: *N.Z. J. Agric. 97:* 491-501.

CORRIGENDA

Vol. 2, p.184, Introduction 2nd para., 4th line:
for 800,000 *read* 8,000.

p.186, Fig. 2 caption, *for* types *read* layers.

p.189, Fig. 4 caption, *for* regeneration *read* plantation.

NEW ZEALAND
JOURNAL OF SCIENCE

Vol. 2, No. 1, March 1959

CONTENTS

Species of *Eriococcus* Targ. (Homoptera, Coccoidea) Associated with the Genus *Leptospermum* Forst. in South-eastern Australia and Tasmania. *J. M. Hoy.*

Contributions to the Quaternary History of the New Zealand Flora. 3. Pollen Analysis of a Peat Profile from Antipodes Island. *N. T. Moar*

Measurement of Flow in a Small Stream. *F. W. Taylor.*

Electric Fishing with Pulsatory Direct Current. *A. M. R. Burnet.*

Marine Fouling at the Port of Auckland. *M. T. Skerman.*

Acetates of Dihydroquercetin (Taxifolin). *H. V. Brewerton* and *R. C. Cambie.*

A Proposed Method of Surveying the Wave State of the Open Ocean. *N. F. Barber.*

The Solubilisation of "Insoluble" Phosphates. 4. The Reaction between Organic Acids and Tricalcium Phosphate. *H. W. Johnston* and *R. B. Miller.*

Observations on the Food of the Hedgehog (*Erinaceus europaeus* L.) in New Zealand. *R. E. Brockie.*

Some Features of the Benthic Environment in Cook Strait. *D. E. Hurley.*

Contributions to a Chromosome Atlas of the New Zealand Flora. 2. Miscellaneous Families. *J. B. Hair* and *E. J. Beuzenberg.*

SUBSCRIPTIONS. Subscriptions for the New Zealand Journal of Agricultural Research will be accepted only on advance payment and only for a full calendar year. Price: 30s. per annum; single copy 5s.; post free (surface mail). Published in alternate months, starting February. Subscriptions should be forwarded to the Publications Officer, Department of Scientific and Industrial Research, P.O. Box 8018, Wellington, New Zealand. Cheques should be made payable to the Department of Scientific and Industrial Research, and if drawn on banks outside Wellington, must have exchange added. Subscribers are requested to give their name and full postal address.

This Journal may also be ordered through The High Commissioner for New Zealand, 415 Strand, London W.C.2, or through any bookseller.

CONTENTS

PAGE

Barley Quality Tests. I. Variety Trials. *Rosa Stern* and *G. M. Wright* 201

Studies on Septoria Leaf-blotch Disease of Wheat (*Triticum aestivum* L.) Caused by *Septoria tritici* Desm. *H. T. Wenham* 208

New Zealand 48s Count Romney Crossbred Wool. *D. A. Ross* 214

Effect on Honey Bees of Thiodan Applied to Broad Beans in a Cage. *T. Palmer-Jones* 229

The Effect of Granule Size on the Efficiency of Granulated Superphosphate Used as a Pasture Topdressing. *F. B. Muller* 234

Construction and Use of Cages in Beekeeping Research. *T. Palmer-Jones* 242

Discovery of the Genus *Basidiophora* Roze et Cornu in New Zealand. *Hasan Jafar* 249

A Note on a Comparison Between the Carcass Composition of Romney and Cheviot Mature Ewes. *A. H. Kirton, R. A. Barton,* and *E. Cresswell* 252

Mineralisation of Nitrogen and Sulphur in Sulphur-deficient Soils. *J. G. White* 255

Housing a Rabbit Colony. *I. J. Cunningham* 259

Trials of Plastic Lining in Glasshouses. *S. Challenger* 262

Photosensitivity Diseases in New Zealand. XV. A Chemical Procedure for the Detection of Facial Eczema Toxicity in Pasture. *D. D. Perrin* 266

A Comparison of the Vitamin B_{12} and Cobalt Contents of Livers from Normal Lambs, Cobalt-dosed Lambs, and Others with a Recent History of Mild Cobalt-deficiency Disease. *E. D. Andrews, L. I. Hart,* and *B. J. Stephenson* 274

The Effects of Season and Weather on the Growth Rate of a Ryegrass and Clover Pasture. *R. W. Brougham* 283

Mathematical Analysis of Growth Curves Replicated in Time. *A. C. Glenday* 297

Seed Disinfection. XV. Glasshouse Tests for Control of Damping-off of Pea Seed. *H. Jacks* 306

Unintentional Introduction of Plants into New Zealand. *A. J. Healy* 312

Rumen Nitrate Metabolism and the Changes Occurring in the Composition of the Rumen Volatile Fatty Acids of Grazing Sheep. *N. D. Jamieson* 314

Root Anatomy of Grasses and Clovers. *Kathleen Soper* 329

Bloat in Cattle. XIII. The Effect of Orally Administered Penicillin on the Fermentation and Foaming Properties of Rumen Contents. *J. L. Mangan, A. T. Johns,* and *R. W. Bailey* 342

Bloat in Cattle. XIV. The Concentration of Soluble Polysaccharide in the Rumen Contents of Cows Fed on Red Clover. *R. W. Bailey* 355

Bloat in Cattle. XV. Further Observations Concerning the Ciliate *Epidinium ecaudatum,* an Inhabitant of the Rumens of Cows Liable to Legume Bloat. *A. E. Oxford* 365

Sticky Traps for Winged Aphids. *R. Close* 375

New Records of Fungous Diseases in New Zealand, 1957–58. *Joan M. Dingley* 380

Concentrations of End-products and Morphological Types of Rumen Bacteria in Silage-fed Sheep. *V. J. Williams* and *K. R. Christian* 387

Yeasts from the Leaves of Pasture Plants. *M. E. di Menna* 394

Fourth Supplement to a "A Revised List of Plants Diseases Recorded in New Zealand" 1957–58. *R. M. Brien* and *Joan M. Dingley* 406

Printed by Coulls Somerville Wilkie Ltd.,
Wellington, New Zealand.